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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

JUNE 1950

ORIGINAL ARTICLES

FOWLER'S SOLUTION AS AN ETIOLOGIC AGENT IN CIRRHOSIS

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and

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The hepatoxic action of arsenic has long been recognized by those using it as a therapeutic agent. In recent years the jaundice which follows parenteral injection of organic arsenical compounds has been recognized as a contaminating viral infection rather than a chemical poisoning. The well known entity of hepatic necrosis, however, coming on soon after parenteral injections of the organic arsenicals results from the toxic action of arsenic upon the liver. Inorganic forms of arsenic likewise cause extensive liver damage in both experimental animals[29,36] and man[1,6,18,21]. Nevertheless, despite the prevalent use of inorganic arsenic as Fowler's solution (potassium arsenite) in leukemia, psoriasis and other dermatologic conditions, the potential danger of such therapy over protracted periods as a cause of chronic liver disease has received very little recognition in recent years. There is need again to stress this fact. It is our purpose in this report briefly to review the literature on the subject and to present 3 cases of portal cirrhosis and arsenical dermatitis resulting from prolonged use of Fowler's solution. The occurrence of acute and chronic hepatic damage caused by organic arsenicals used therapeutically and the accidental ingestion or contact with inorganic arsenicals has been well covered in older and recent reports[1,2,6,8,18,21,26,28,29]. We will discuss only the role of inorganic arsenic administered therapeutically as an etiologic agent in portal cirrhosis.

Bang[3] in 1774 and Geyer[10] in 1898 both reported the occurrence of general anasarca and ascites following the ingestion of arsenic. In 1895 Hutchinson[16] reported poisoning from the use of Fowler's solution administered over an 8 year period. His patient developed discoloration of the skin, keratosis of the palms and soles and ascites for

which paracentesis was performed on 3 occasions. Discontinuation of the arsenic therapy was followed by complete recovery. Hamburger[12] in 1900 reported a similar case due to protracted use of Fowler's solution. In the same year Sturrock[32] and Reynolds[23] both reported chronic hepatitis in persons who had been poisoned by contaminated beer and whose livers showed much damage at post mortem. Broadbent[5] in 1903 described the occurrence of ascites in a patient with Hodgkin's disease treated with an oral arsenical. It was his belief that the patient had "arsenical cirrhosis of the liver, spleen, and lungs". In 1921 Stockman[31] described ascites and hepatomegaly in one of his patients who had been taking *liquor arsenicalis* over a period of 6 years. In 1928, O'Leary, Snell and Bannick[19] cited 2 cases of portal cirrhosis developing after long continued use of Fowler's solution. These cases of chronic arsenical poisoning had arsenical dermatitis and the findings typical of portal cirrhosis. In one an abdominal exploration disclosed a hobnailed liver.

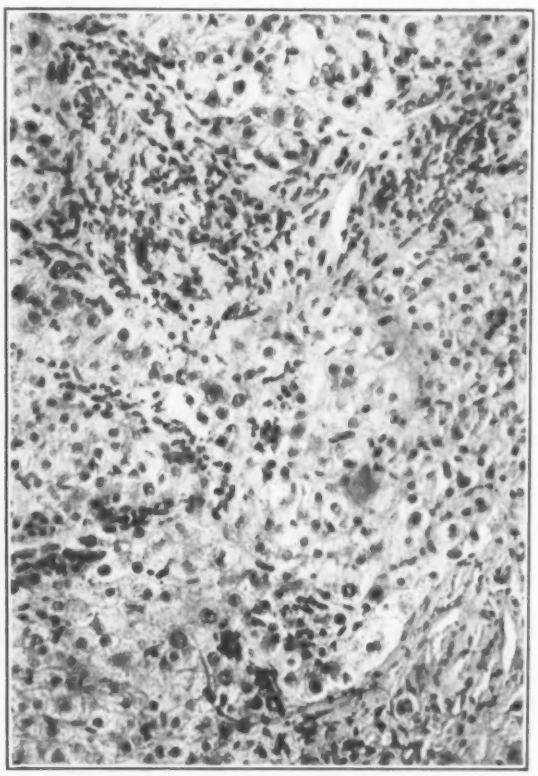

FIG. 1.—Sections of liver biopsy in Case 1 showing chronic parenchymal damage, increased fibrous and mesenchymal reaction.

It was their opinion that such cases represented a clinical entity which they called arsenical cirrhosis. In 1930 Weir[37] recorded 2 cases of portal cirrhosis resulting from continued use of Fowler's solution. In contradistinction to the cases of O'Leary, Snell and Bannick where therapy appeared to be of

definite benefit, Weir reported progression of the disease with treatment in 1 case and some improvement in his second.

Case Reports. CASE 1. A man, aged 57, was admitted to the University Hospitals in July, 1945, with a history of myelogenous leukemia having been diagnosed by his physician 2 years prior to admission. He was placed on Fowler's solution, 10 drops 3 times daily and iron therapy. He also received 7 or 8 Roentgen-ray treatments over a year's time. Nine months prior to admission, he noticed abdominal swelling, ankle edema and a coppery pigmentation of the skin. His abdomen was tapped on several occasions. Three months prior to admission, his skin became dry and scaly. There was no history of alcoholism.

Physical examination disclosed a chronically ill patient with speckled pigmentation of the trunk and abdomen as well as typical arsenical keratosis of the palms and soles. The sclerae were slightly icteric. The abdomen was moderately distended with ascitic fluid and the liver and spleen were both palpable 6 cm. below the costal margins. Edema of the ankles was present and some freely movable pea-sized glands were palpated in the submaxillary and axillary regions. Blood and bone marrow studies confirmed the diagnosis of chronic myelogenous leukemia. Total serum proteins were 4.6 gm., albumin 2.4 gm., globulin 2.03 gm., and fibrinogen 179 mg. per 100 cc. The cephalin flocculation test was 3+ at the end of 48 hours. Hippuric acid excretion was normal. Total cholesterol was 100 mg. per 100 cc. and prothrombin time was 41 seconds

FIG. 2.—Esophagram showing esophageal varices in Case 2.

with a normal of 32 seconds. Urinary arsenic was 1.68 mg. per liter (normal in this laboratory being 0–.06 mg. per liter). The van den Bergh reaction was 4.6 mg. of total bilirubin per 100 cc. A liver biopsy revealed acute and chronic parenchymal liver disease with increased periportal fibrosis extending interlobularly. Roentgen-ray of the chest and esophagograms were negative. Diagnoses of chronic myelogenous leukemia, chronic arsenical poisoning with liver degeneration and exfoliative dermatitis were made. The patient was placed on a high carbohydrate diet, mercurial diuretics and Roentgen-ray therapy for his leukemia. He required several paracenteses before being discharged to the care of his local physician.

He was readmitted to the hospital in February, 1946, in the terminal stages of leukemia with no change in his liver and skin conditions. Despite Roentgen-ray and transfusion therapy, he died 3 weeks after admission. No autopsy was done.

Case 2. A farmer, aged 56, was first observed at the University Hospitals in November, 1936, with an itching and vesicular skin lesion which was diagnosed as dermatitis herpetiformis. Except for this and bilateral inguinal hernias, which were repaired, the history, physical examination and laboratory findings were negative. He was discharged and placed on Fowler's solution, 10 drops 3 times daily, and told to report to his local physician for periodic check-ups. He was next seen in November, 1939, stating that he had been taking his medication in the interim and had several remissions and exacerbations of his skin complaints. He had never been completely free from his skin lesion or pruritus. The physical findings upon admission were similar to those found previously and the diagnosis of dermatitis herpetiformis was made again. He was told to continue on Fowler's solution, 8 drops 3 times daily, inasmuch as he believed that it had helped him, but was again warned to have his family physician evaluate his progress and his medication. He was next seen in July, 1942, stating that the medication had given him great relief and therefore he had continued taking it except for a period of 3 months. His only complaints were the gradual onset of abdominal and leg swelling and dyspepsia 3 weeks prior to the present admission.

Physical examination revealed generalized mottling and bronzing of the skin with keratosis of the palms and soles. The liver was palpable 2 fingers' breadth below the right costal margin and was not tender. The abdomen was distended with fluid. The remainder of the physical examination was negative. Laboratory studies showed normal urinary findings, negative chest Roentgen-rays and electrocardiograms. The cephalin flocculation test was normal, alkaline phosphatase was 6.2 Bodansky units. Chemical examination of the nails and hair was negative for arsenic. Esophagograms revealed the presence of varices. A diagnosis of arsenical cirrhosis was made and the patient was placed on a high carbohydrate, low fat diet and upon Theocalcin. After a few days in the hospital, he was discharged and did well until February, 1945, when he again developed gradual swelling of his abdomen and legs which necessitated his readmission to the hospital. Arsenical keratosis of the hands and feet was diagnosed by members of the Dermatology Department. Ascites and marked pitting edema of the lower extremities were present. The liver was no longer palpable. Laboratory findings revealed a red cell count of 3,640,000; leukocytes, 4,100 (36% neutrophils, 4% eosinophils, 2% basophils, 54% lymphocytes and 2% monocytes). The Wassermann reaction was negative. Urinary examination revealed a specific gravity of 1.012, traces of albumin, many red blood cells and a few hyaline casts. Urea clearance was 43% of normal. Blood urea nitrogen was 20 mg. and treatment 1.5 mg. per 100 ml., creatinine was 1.5 mg.; the van den Bergh test, galactose tolerance and hippuric acid excretion tests were normal. Total protein was 6.0 gm., albumin 3.6 gm., and globulin 2.4 gm. per 100 ml. Roentgenograms of the chest and electrocardiograms were normal. Esophageal varices were demonstrated by Roentgen-ray. Under therapy consisting of bed rest and mercurial diuretics, his edema and ascites subsided but at the time of discharge the urine still showed a trace of albumin and some red blood cells. We have attempted on several occasions to trace the patient but have not heard from him since his discharge in 1945.

Case 3. A farmer, aged 57, was admitted to the University Hospitals in May, 1948, complaining of bloating of 8 months' duration and of "liver trouble" for a similar length of time. He had psoriasis for which he had been taking 3 to 8 drops of Fowler's solution 3 times daily for at least 2 years prior to hospital admission. In April, 1943, he first noted pain and bloating in his lower abdomen after meals. He also became aware of an eversion of his umbilicus and of ankle edema at this time. In July he was hospitalized elsewhere and after having a series of gastrointestinal and gallbladder roentgenograms was told he had "liver trouble" and that his liver was

enlarged. He was placed on a low fat diet and discharged 3 weeks prior to his present admission which followed an episode of hematemesis and tarry stools. At about this time he began noticing an enlargement of his abdomen. There was no story of any alcoholic intake.

Physical examination disclosed a chronically ill white man with a brown mottled muddy pigmentation over the anterior chest wall and lumbar region. The skin was scaly and there was a warty keratosis of the palms and soles. The abdomen was distended with ascitic fluid, and mild edema of the ankles was present. The liver could not be palpated. The remainder of the examination was noncontributory. Laboratory findings showed a normal urine. The blood contained 5 gm. of hemoglobin per 100 ml., 2 million red cells and 10,000 white cells, with a normal differential count. Bleeding, coagulation and prothrombin times were all normal. The quantitative van den Bergh, thymol turbidity, cephalin flocculation, zinc sulphate precipitation and bromsulphalein tests were normal. Total proteins were 6.51 gm. per 100 ml. (albumin 3.67 gm., and globulin 2.84 gm.). Roentgen-ray of the chest revealed mild pulmonary fibrosis. Roentgenograms of the esophagus, stomach, duodenum and colon were all normal. The diagnosis of portal cirrhosis and arsenical dermatitis was made and the patient was placed on a high carbohydrate, high protein, low fat diet, fortified with brewer's yeast, choline citrate and ferrous sulphate. His abdomen was tapped and 6 liters of clear fluid were withdrawn. He showed a satisfactory diuresis to intramuscular mercurial diuretics. The patient was discharged in care of his family physician who notified us of his death 5 months after leaving the hospital.

Discussion. According to Goodman and Gilman[11], the arsenical compounds used therapeutically are best classified with respect to their chemical composition. They can thus be divided into trivalent and pentavalent and into inorganic and organic arsenicals. The inorganic arsenicals are more toxic and are generally employed for their effects upon tissues of the body. Potassium arsenite (Fowler's solution) is the *inorganic* arsenical most frequently used for medicinal purposes and contains arsenic in its trivalent form. The drug is used chiefly in the therapy of leukemia and in various dermatologic conditions. The less toxic *organic* arsenicals are also in trivalent and pentavalent forms. The trivalent organic arsenicals Mapharsen and Arsphenamine are highly treponemicidal. The representative pentavalent organic arsenicals such as carbarsone, acetarsone and tryparsamide are employed primarily against protozoan parasites, such as ameba and trypanosomes. The organic arsenicals mentioned are stable and contain a benzene ring to which the arsenic is attached. They are active in their organic forms. Sodium cacodylate is an organic compound in which the arsenic is attached to an aliphatic radical and its activity depends upon release of inorganic arsenic. Its use is similar to that of Fowler's solution.

Arsenic is a protoplasmic poison affecting most tissues of the body. Its toxic manifestations therefore become evident in the gastrointestinal tract, circulatory system, kidney, blood, central nervous system, skin and liver. We are especially concerned here with the effect of prolonged oral ingestion of Fowler's solution (potassium arsenite) upon the liver and skin. All 3 of our patients exhibited typical arsenical dermatitis and keratosis. This usually consisted of a mottled dark brownish pigmentation of the skin of the trunks and extremities giving a raindrop effect. The skin was dry and often exfoliated and there was an associated keratosis of the palms and soles. Arsenic is deposited in the hair and nails and can be found there often after all traces have disappeared from the urine. Carcinogenic properties of arsenic as a factor in carcinoma of the skin constitute a well recognized occupational hazard to those working with arsenic[13,14].

The liver bears the brunt of arsenic damage[35]. The acute hepatic necrosis caused by parenteral organic arsenicals and inorganic arsenicals has been men-

tioned. The liver may be especially vulnerable to prolonged oral intake of small amounts of inorganic arsenic such as Fowler's solution. Sensitivity to arsenic need not be postulated, although it is known that certain persons are intolerant of even small doses of arsenic in any form[6,35]. The soluble arsenic salts are easily absorbed from the gastrointestinal tract and after entering the blood stream are deposited in the liver, kidney, spleen and intestines. With parenteral organic arsenical injection much more arsenic is lost in the urine than with oral ingestion of inorganic salts[11]. Weir and Raiziss[37] have found that as a depot for arsenic storage, the liver comes first in point of concentration. Thus greater amounts of arsenic are removed from therapeutic arsenicals which are orally ingested and carried by the portal blood than by arsenic introduced parenterally. Frenkel-Heiden and Navassart[9] found the elimination of arsenic in the feces about 8 times as great as in the urine, presumably through reabsorbtion from the bile. It may take from 10 days for complete elimination of arsenic after a single parenteral dose, and up to 70 days after repeated administration[11]. This slow excretion is the basis for the cumulative toxic action of arsenic. Thus it is evident why prolonged medication with Fowler's solution may cause chronic liver damage which may eventually culminate in cirrhosis.

Broadbent described intralobular and intercellular cirrhosis as the lesions found in his case. Sollman[30] and Ullman[33,34] described toxic fatty changes, most marked centrally as the acute effects of arsenic on the liver. According to O'Leary, Snell and Bannick these fatty degenerative and necrotic lesions may eventually be replaced by fibrous tissue resulting in cirrhosis. The liver biopsy performed on one of our cases revealed chronic and acute parenchymal damage with increased periportal and interlobular fibrosis[4]. Esophagograms in another case revealed esophageal varices[17]. The liver function tests, in the patient who was slightly jaundiced, were abnormal. The 2 patients without jaundice had essentially normal liver function. The frequent lack of functional impairment as determined by liver function tests in non-jaundiced cirrhotic patients is well recognized[20,24].

The patients of Hamburger and Hutchinson both improved on discontinuation of the toxic agent in the therapeusis. The 2 patients of O'Leary, Snell and Bannick both did well on avoidance of arsenic when given saline purgatives, diuretics, and restricted fluid intake. They also advised use of sodium thiosulphate. Upon essentially similar management, one of Weir's patients showed no improvement, whereas the other showed some improvement. In our series, one of our patients responded well to management consisting of high protein, high carbohydrate, low fat diet, mercurial diuretics, vitamins, brewer's yeast and choline. However, 5 months later his private physician informed us of his death. Our 2nd patient died of his leukemic condition and therapy had not apparently improved his cirrhosis. Our 3rd patient showed loss of ascites and clinical improvement on bed rest, restrictive fluids, high carbohydrate diet and mercurials. However, 3 years later he had recurrence of his ascites which again responded to therapy, but unfortunately the patient was then lost track of. The results of therapy in all such cases are difficult to assess, just as they are in any case of cirrhosis because of spontaneous and therapeutically induced remissions. None of the cases reported in the literature was followed long enough to state that the patient was definitely cured. It is doubtful whether the fibrotic cirrhotic process is ever reversible. We believe

that patients exhibiting arsenical cirrhosis should be treated as those with any other form of portal cirrhosis in addition to the elimination of the arsenic. British Anti-Lewisite (BAL) might be given a trial, although it probably would not have any effect upon the fibrotic processes but might counteract some of the toxic arsenical action upon the liver cells and tissues[22].

Arsenic in the form of Fowler's solution at one time enjoyed considerable use as a tonic and was extensively used in various forms of anemia. It was not of much benefit in either of these conditions and fell into disrepute. Its use in leukemia, psoriasis and other dermatologic conditions has to a great extent been replaced by other more efficacious and less toxic forms of therapy. Nevertheless chronic arsenic poisoning due to medicinally administered arsenic is still seen all too frequently, for many physicians do not appreciate the cumulative toxic action upon liver and other organs of small doses of arsenicals given over a long period of time.

Summary. 1. The occurrence of portal cirrhosis and ascites following chronic arsenic poisoning due to prolonged administration of Fowler's solution (potassium arsenite) is discussed.

2. The literature is reviewed briefly and 3 cases of arsenic cirrhosis are presented.

3. The hepatotoxic action of arsenic is discussed and the potential dangers to liver of prolonged administration of Fowler's solution is pointed out.

Since submission of this report, we have seen one more case of cirrhosis following prolonged treatment with Fowler's solution. The patient was a 43 year old male who had been treated for psoriasis with Fowler's solution over a period of years. There was no history of any alcoholic intake or any other etiologic factor in the causation of his cirrhosis. The physical and laboratory findings were typical of cirrhosis with marked hepatic damage. Evidence of arsenical dermatitis and keratosis was present. The patient died in cholemia, and post mortem examination revealed a post necrotic cirrhosis with portal vein thrombosis.

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STUDIES WITH THE QUANTITATIVE CEPHALIN-CHOLESTEROL FLOCCULATION REACTION. I. EFFECT OF TEMPERATURE VARIATION. SERUM PROTEIN PATTERNS IN LIVER DISEASE*†

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It is generally recognized from the work of Whipple and Madden[15], that the liver plays an important role in the formation of plasma proteins, although one or more of these proteins may have a secondary site of origin in other tissues or organs. The work of Schoenheimer and Rittenberg[22] and others with stable or radioactive isotopes as tracers, have shown that a dynamic equilibrium exists between the circulating plasma proteins and the proteins present in tissues which they supply with nutrients. The more recent views on this subject have been stated in an article by Cannon[2]. It is apparent that any marked disturbances in the functioning of normal liver cells, or in the enzyme systems within these cells, will be reflected by changes in the composition of the plasma proteins elaborated within these cells. While these changes are most pronounced in those diseases which directly involve the liver cells (*e.g.*, infectious hepatitis) similar changes in the plasma proteins occur in many diseases which are viral or bacterial in origin (*e.g.*, bacterial endocarditis). Maclagan[14] has suggested that these changes in plasma proteins may reflect an increase in circulating antibody.

Although it is difficult to show any changes in the albumin-globulin ratio by the usual classical methods of protein separation, except in the very late stages of a disease, Dauphinee and Campbell[3] have reported the presence of an abnormal globulin, using chemical separation, in the serum of patients with diseases which affect primarily the parenchymal cells. Electrophoretic studies of serum proteins in hepatic disease[7,11,18] have shown that there is usually a decrease in the albumin fraction with an accompanying increase in the globulin fraction, especially in the gamma globulin.

The simplest and most clinically useful methods used to determine these protein changes in disease sera, as compared to normal sera, have been the numerous serum protein flocculation and turbidity tests. Of these the most widely used and best studied have been the cephalin-cholesterol flocculation test of Hanger[9] and the thymol turbidity test of Maclagan[13]. In a recent article, Maclagan[14] has discussed the underlying chemical factors for a num-

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† A preliminary report of this work was published in the Bull. N. Y. Acad. Med., **25**, 456, 1949.

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ber of these flocculation tests in terms of the electrophoretic protein fractions involved, either as active substances or as inhibitors. Two major drawbacks to these simple flocculation tests have been that the degree of flocculation is expressed qualitatively (*i.e.*, 0 to 4 +), and that since a large number of diverse diseases may give slight flocculation, little significance is attached to values less than 2 +. A number of experimenters have attempted to overcome these difficulties by making use of the experimental facts that simple dilution and, or, temperature variation will detect differences in protein composition which might not otherwise be apparent. Makari[16] has made use of a technique involving serial dilution of sera with saline in conjunction with the cephalin-cholesterol method. With this procedure he is not only able to differentiate normal from disease sera but to obtain different curves for different classes of disease, *e.g.*, to distinguish between liver disease and reticulo-endothelial disturbances. Neefe and Reinhold[19] were the first to report the effect of temperature on the Hanger reaction, stating that flocculation generally occurred more rapidly and completely at 37.5° C. than at room temperature. Frisch and Quilligan[6] have used both serial dilution and temperature effect, together with a modified cephalin-cholesterol emulsion, to distinguish between normal and liver disease sera. Saifer[20] has employed a quantitative cholesterol method for the determination of the total cholesterol content of the centrifuged flocculated material from the Hanger test. Since this method can determine slight variations in the amount of flocculated material in terms of *quantitative* cholesterol units, it has been employed

TABLE 1.—EFFECT OF VARIATION OF TEMPERATURE ON THE CEPHALIN–CHOLESTEROL FLOCCULATION REACTION WITH NORMAL SERA

	Cholesterol Unit Values				
Case	*4° C*	*25°C*	*37° C*	*56° C*	*Remarks*
1	327(4+)	47(−)	73(−)	611(4+)	
2	460(2+)	78(−)	144(1+)	568(4+)	Recovered from hepatitis
3	370(2+)	81(−)	75(−)	563(3+)	
4	167(1+)	20(−)	113(−)	553(3+)	
5	541(2+)	102(−)	323(1+)	642(4+)	
6	144(−)	59(−)	504(3+)	512(4+)	
7	309(2+)	65(−)	61(−)	349(2+)	
8	214(−)	5(−)	29(−)	228(2+)	
9	163(1+)	22(−)	24(−)	459(4+)	
10	502(2+)	72(−)	89(−)	518(4+)	
11	490(3+)	15(−)	39(−)	508(4+)	
12	348(2+)	39(−)	28(−)	459(4+)	
13	497(3+)	63(−)	79(−)	547(4+)	
14	131(−)	16(−)	10(−)	542(3+)	
15	246(1+)	9(−)	2(−)	542(3+)	
16	326(2+)	42(−)	23(−)	485(4+)	
17	320(2+)	52(−)	180(2+)	464(4+)	
18	126(2+)	21(−)	112(2+)	474(4+)	
19	146(1+)	30(−)	35(−)	417(3+)	
20	98(1+)	8(−)	3(−)	351(3+)	
21	269(2+)	36(−)	82(1+)	620(4+)	Recovered from hepatitis
22	337(2+)	94(−)	494(2+)	615(4+)	Recovered from hepatitis
23	209(1+)	43(−)	93(1+)	615(4+)	
24	40(−)	2(−)	2(−)	356(3+)	
25	210(3+)	32(−)	70(1+)	380(4+)	
26	199(1+)	1(−)	17(−)	373(2+)	

to study the effect of a wide range of temperature variation on the Hanger reaction with both normal and disease sera. The studies presented here confirm the views of previous investigators that different protein patterns are obtained with disease sera as compared to normal sera. But in addition with these quantitative techniques it becomes possible to detect variations in protein composition at different stages in the *same* disease (*e.g.*, infectious hepatitis) as well as for different diseases.

Procedure. The procedure is exactly as described for the quantitative cephalin-cholesterol method[20] except that each unknown serum was pipetted into 4 separate centrifuge tubes, 1 for each temperature. A saline blank, a normal serum and a known positive serum were also prepared in quadruplicate, 1 for each of the 4 temperatures. One set of tubes, including unknowns, a blank, a normal serum and a positive serum, was placed in an ice box at 4° C. Another set was kept at 25° C. in a closed water bath. A third set was kept at 37.5° C. in a closed water bath and a fourth set was kept at 56° C. in another water bath. The tubes were kept at constant temperature for 24 hours and then centrifuged for 15 minutes at 3000 r.p.m. The cholesterol unit value for each serum at the 4 different temperatures were determined exactly as described in previous publications[20,21].

Results. A. NORMAL SERA. Some 26 sera from known normal subjects of all age groups and both sexes were run

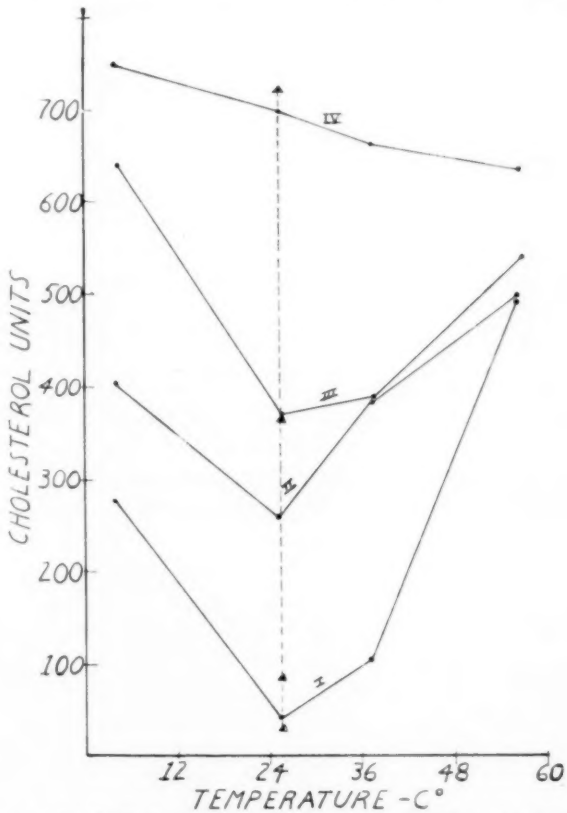

FIG. 1.—Composite graph of all the average cholesterol unit values obtained for each protein pattern (*i.e.*, Types One to Four). The △ points on the dotted line indicate the transition cholesterol unit values at which the type changed during the serial study of a case of acute infectious hepatitis.

by the procedure given above. The results obtained in these runs, expressed in cholesterol units, are given in Table 1. These results substantiate the previously published findings that normal sera when run at room temperature (20° to 25°) give cholesterol unit values of 103 or less with the average value for the 26 cases being 39.6 units. These results also prove conclusively the importance of temperature control for the Hanger method. In agreement with Neefe and Reinhold[19] the most consistent results for normal sera were obtained at 25°C. Variation of temperature in either direction generally caused an increase in the degree of flocculation.

These results show that for normal sera quantitative cholesterol unit values of less than 103 units are obtained at 25°C. with an increase in the cholesterol unit value both at 4°C. and 56°C. above that obtained at 25°C. but with the greater increase occurring at 56°C.

The normal serum protein pattern (Type 1) was obtained by plotting the average cholesterol unit values against temperature and is shown in Fig. 1.

B. Chronic Infectious Hepatitis. The 7 cases shown in Table 2 represent the results obtained with the sera of patients who have presumably recovered from liver disease, but which gave consistently 1 + to 3 + Hanger reactions on repeated analyses as well as positive findings with other liver function tests.

Except for an increase in the cholesterol unit value at 25°C. above 100 units, the effect of variation of temperature in these cases is the same as for normal sera.

TABLE 2.—EFFECT OF VARIATION OF TEMPERATURE ON THE CEPHALIN-CHOLESTEROL FLOCCULATION REACTION WITH SERA FROM CASES OF CHRONIC HEPATITIS

	Cholesterol Unit Values				
Case No.	*°° C.*	*25° C.*	*37° C.*	*56° C.*	*Remarks*
201	330(3+)	197(2+)	320(3+)	434(3+)	Recovered from hepatitis
202	281(3+)	146(2+)	—	460(3+)	Recovered from hepatitis
203	183(1+)	176(1+)	321(1+)	503(4+)	History of jaundice
204	320(2+)	180(2+)	168(2+)	437(4+)	History of jaundice
205	222(2+)	134(−)	134(−)	388(3+)	History of jaundice
206	388(3+)	222(2+)	148(2+)	411(4+)	History of jaundice
207	406(1+)	124(1+)	174(1+)	510(3+)	History of liver disease

TABLE 3.—EFFECT OF VARIATION OF TEMPERATURE ON THE CEPHALIN-CHOLESTEROL FLOCCULATION REACTION WITH HEPATITIS SERA

	Cholesterol Unit Values				
Case	*4° C.*	*25° C.*	*37° C.*	*56° C.*	*Remarks*
301	647(4+)	517(3+)	472(3+)	606(4+)	Sample taken 5.4.48
301*a*	705(4+)	638(3+)	582(3+)	699(4+)	Same case — Sample taken 5.27.48
302	633(3+)	577(3+)	508(3+)	596(4+)	Sample taken 5.10.48
302*a*	813(4+)	626(3+)	659(4+)	718(4+)	Same case — Sample taken 5.18.48
303	553(4+)	397(4+)	529(4+)	427(4+)	
304	667(4+)	346(1+)	431(2+)	530(3+)	
305	640(4+)	238(1+)	170(−)	437(3+)	
306	655(4+)	399(2+)	413(3+)	510(3+)	
307	600(3+)	362(2+)	366(2+)	558(4+)	
308	579(2+)	216(1+)	189(−)	505(3+)	
309	486(4+)	368(3+)	291(3+)	414(4+)	Sample taken 6.22.48
309*a*	469(4+)	266(2+)	440(2+)	358(3+)	Sample taken 6.29.49

These cases may be considered as representing a residual or slight amount of continuing parenchymal cell injury. The serum protein patterns (Type 2) are obtained by plotting average cholesterol unit values against temperature and are also shown in Fig. 1.

C. Infectious Hepatitis. A group of 12 cases of infectious hepatitis was then selected for study with the above procedure and the results obtained in these runs are given in Table 3. It should be emphasized that all the cases listed in this table had been hospitalized for some time and had undergone intensive therapeutic treatment for a number of weeks previous to the time that these tests were performed. Although some of these cases were jaundiced, other chemical findings, including the A/G ratio, were essentially normal except for the total bilirubin and thymol turbidity determinations. This group of cases can therefore be said to represent patients having a moderate degree of liver damage as shown both by the clinical evidence and the biochemical findings.

The results obtained in these runs show that for these cases of infectious hepatitis the cholesterol unit values at 25°C. is usually above 100 units and that there is an increase in the cholesterol unit values both at 4°C. and 56°C. above that obtained at 25°C. but with the greater increase occurring at 4°C.

The average cholesterol unit values for these cases when plotted against temperature give a protein pattern (Type 3) which is representative of cases with a moderate degree of liver damage.

D. Cirrhosis. The 8 cases of liver disease shown in Table 4 represent the most severe cases with permanent liver damage present at our institution. All of these cases were clinically diagnosed as diffuse nodular cirrhosis. The diagnosis in most cases was confirmed either at autopsy or by liver punch biopsy. In addition the positive biochemical findings in these cases included high bromosulfalein retention, inverted A/G ratios, and low cholesterol ester ratios.

TABLE 4.—EFFECT OF VARIATION OF TEMPERATURE ON THE CEPHALIN–CHOLESTEROL FLOCCULATION REACTION WITH CIRRHOSIS SERA

	Cholesterol Unit Values				
Case	*4° C.*	*25° C.*	*37° C.*	*56° C.*	*Remarks*
401	861(4+)	816(4+)	801(4+)	738(4+)	Biopsy indicates cirrhosis 5.14.48
401*a*	686(4+)	642(4+)	625(4+)	552(4+)	Same case 6.10.48
402	791(4+)	700(4+)	630(4+)	628(4+)	
403	837(4+)	784(4+)	792(4+)	717(4+)	
404	745(4+)	690(4+)	706(4+)	643(4+)	Biopsy indicates cirrhosis 6.3.48
404*a*	561(4+)	549(4+)	551(4+)	488(4+)	Same case 6.17.48
405	796(4+)	758(4+)	539(4+)	680(4+)	
406	726(4+)	677(4+)	686(3+)	666(4+)	

The data for the cirrhosis cases show that the average cholesterol unit values obtained at 25°C. are usually about 7 times higher than the maximum value for normal sera and that while there is an *increase* in the cholesterol unit value obtained at 4°C. over that obtained at 25°C., there is a decrease at 56°C. as compared to the 25°C. value.

The average cholesterol unit values for these cases when plotted against temperature give protein patterns (Type 4) which are representative of the most severe kind of liver damage, that is, cirrhosis.

E. Infectious Mononucleosis, Malaria. In addition to the liver disease cases discussed above, some 11 other cases, diagnosed clinically as infectious mononucleosis or malaria, were run by the described procedure (Table 5). It was found experimentally that the protein patterns obtained were identical with those described for liver diseases, that is, Types 2, 3, or 4, and that this test could *not* be used to distinguish these diseases, from either hepatitis or cirrhosis.

F. Serial Studies on a Single Case of Acute Infectious Hepatitis. The values shown in Table 6 represent serial studies of a single case of acute infectious hepatitis from the peak of the disease at the time of admission until a week after discharge when the patient had resumed full normal activity.

Analysis of the results given in Table 6 lead to the following conclusions:—1) That the cholesterol unit values at 25° provide the most *sensitive* index of the clinical condition of the patient. 2) That Type 4 patterns are *not limited* to cases of cirrhosis but are also found in hepatitis when the damage to the liver is severe enough. 3) While the cholesterol unit value at 25° represents

TABLE 5.—EFFECT OF VARIATION OF TEMPERATURE ON CEPHALIN-CHOLESTEROL FLOCCULATION REACTION WITH SERA FROM CASES OF MALARIA AND INFECTIOUS MONONUCLEOSIS

Case	*Cholesterol Unit Value 25°*	*Type of Pattern Obtained*	*Diagnosis*
601 (6.22)	480(4+)	4	Infec. Mononuc.
601*a*(7.1)	324(2+)	2	" "
602 (6.29)	415(3+)	4	" "
602*a*(7.1)	348(3+)	3	" "
602*b*(7.6)	296(2+)	3	" "
602*c*(7.24)	123(−)	2	" "
608	178(−)	2	" "
604	110(−)	2	" "
605	745(4+)	4	Malaria
605*a*	696(4+)	4	Malaria
606	647(4+)	4	Malaria

TABLE 6.—SERIAL STUDIES OF THE EFFECT OF VARIATION OF TEMPERATURE ON THE CEPHALIN-CHOLESTEROL FLOCCULATION REACTION WITH A CASE OF ACUTE HEPATITIS

Date	*Pattern Obtained*	*Cholesterol Unit Value 25°*	*Other Liver Function Tests*
6.8.48 (admitted)	4	723	I. I.—57 Tot. Bil.* 7.5
6.10.48	4	624	I. I.—43
6.14.48	4	581	I. I.—31 Tot. Bil.—6.0
6.17.48	4	483	I. I.—26.4 Tot. Bil.—1.6
6.22.48	3	368	I. I.—17 Tot. Bil.—1.2
6.24.48	3	355	I. I.—16 Tot. Bil.—1.2
6.29.48	3	266	I. I.—20 Tot. Bil.—1.0
7.8.48	2**	84	I. I.—12
7.12.48	2**	58	I. I.—17 Tot. Bil.—0.9
7.20.48 (discharged)	1	26	I. I.—12 Tot. Bil.—0.7
7.27.48†	1	32	I. I.— 7 Tot. Bil.—0.2

* Total bilirubin expressed in mg. per cent was performed by the method of Malloy and Evelyn.[17]

** This pattern was grouped as Type 2 instead of Type 1 as the cholesterol unit value was about 2 to 3 times this patient's normal value.

† This run was performed after a week's normal activity.

quantitative changes in the serum proteins as elaborated by the liver, the protein patterns represent *qualitative* changes in the protein composition which change only when certain critical clinical levels in the condition of the patient are reached. This is clearly indicated by the composite plot of *all* the average curves for the liver disease protein patterns, that is, Type 1 to 4, as shown in Fig. 1. On this *plot* is superimposed the transition cholesterol unit values at 25°C. obtained in the serial studies at which the protein pattern changes from one type to the next. 4) The last 2 sets of values indicate that each individual maintains a fairly constant cholesterol unit value at 25°C. when in good health.

Discussion. In these studies temperatures of 4°, 25°, 37.5°, and 56°C. were chosen mainly because they were conveniently available laboratory temperatures. Temperatures above 56° were not used because the cephalin-cholesterol emulsion in the saline blank flocculated out of solution within 24 hours at these higher temperatures.

In general the results obtained with these temperature studies agree with the findings of Neefe and Reinhold[19] in that increased flocculation usually occurs at 37.5°C., but disagree with those of Frisch and Quilligan[6] in that increased flocculation with normal sera occurs at 4°C. as well as at 56°C. However it should be pointed out that the emulsion used by the latter authors was prepared at room temperature rather than at 65° to 70° as recommended by Hanger[9] and might react quite differently to changes in temperture.

The data shown in Fig. 1 indicate that the greatest differences in the degree of flocculation between normal and disease sera are obtained at 25°C. rather than at higher or lower temperatures. These results provide experimental justification for the routine use of room temperature, that is, between 20° to 25°C., for the Hanger test although it would be preferable to allow the tubes to stand in a constant temperature water or incubator which is maintained at 25° ± 1°C to insure constancy of results. The results obtained at 37.5°C. usually differ only slightly from those obtained at 25°C., so that little importance is attached to the cholesterol unit values obtained at this temperature in the interpretation of the clinical data in this paper. For routine clinical work, determinations at 37.5°C. may be eliminated.

The regular Hanger antigen was used in these studies because of its almost universal use in routine clinical laboratories and because of the numerous published papers which have attested to the clinical validity of the method. The quantitative method provides the added advantage of providing a convenient means of measurement of differences which cannot be observed with the original qualitative method.

Electrophoretic studies of normal or diseased serum proteins will generally provide more complete information as to changes in the various protein fractions than can the procedures described in this paper, but such methods are excluded from most clinical laboratories both by the cost of the instrument and its inadequacies for routine clinical work. However the electrophoretic studies of Gray and Barron[7], Kabat, *et al.*[11], and Moore[18] have established the fact that the changes in the serum proteins as a result of liver disease occur mainly in the albumin and gamma globulin fractions. According to the work of Moore *et al.*[18] and Hanger[10] the mechanism of a positive cephalin-cholesterol flocculation test is such that the gamma globulin fraction attaches itself to the emulsion when there is a *relative* increase in gamma globulin

content over an inhibitory substance present in the electrophoretic albumin fraction. So that in essence the cephalin-cholesterol flocculation reaction is a simple chemical method for demonstrating the *same* changes in the composition of the serum proteins which can be shown electrophoretically. The results presented in this paper carry this analogy to the electrophoretic method one step further in that they permit the subdivision of human sera into 4 distinct types with respect to their protein patterns and which in turn may be characteristic of the degree of liver damage.

The method described in this paper possesses a number of important advantages over the original Hanger method. Firstly, the subdivision of liver disease into protein patterns based on the severity of the disease may serve to indicate when a patient with clinical or biochemical signs of liver disease is in need of active hospitalization or treatment. Such a differentiation is especially important in view of the fact that more than 10% of all cases of viral hepatitis develop persistent symptoms of liver damage after apparent clinical recovery. The literature dealing with the "chronic hepatitis syndromes" and its possible relation to cirrhosis has recently been discussed in an article by Brick[1].

Secondly, the type of protein pattern obtained may serve to differentiate liver disease cases from other diseases which give positive cephalin-cholesterol flocculation reactions. Hanger[9] has shown that diseases such as obstructive jaundice, rheumatoid arthritis, tuberculosis, bacterial endocarditis, give positive cephalin-cholesterol flocculation tests. When sera from such cases were determined with the above described quantitative procedure either Type 2 patterns were obtained or a new pattern which differed from any of those obtained for liver disease. These results are not included in this paper because of the difficulty in excluding from the primary diagnosis some degree of liver damage as a secondary factor resulting from the more chronic condition.

A number of investigators have pointed out that both malaria[8,12] and infectious mononucleosis[4,5] are often accompanied with demonstrable impairment of the parenchymal cells which resemble early infectious hepatitis. In this connection it is interesting to note that the types of protein patterns obtained with sera from these diseases (which give positive Hanger reactions) are identical with those obtained in liver disease.

Summary. 1. The effect of temperature on the Hanger reaction has been studied using a large number of normal and abnormal (that is, liver disease) sera by means of the quantitative cephalin-cholesterol flocculation reaction. There is a marked variation in the amount of flocculation obtained with the same serum at different temperatures. The necessity of performing the Hanger reaction at a constant temperature (25°C.) is stressed.

2. These temperature studies indicate that all human sera with suspected liver disease can be divided into 4 serum protein patterns based on the cholesterol unit values obtained at 4°, 25°, 37.5° and 56°. These patterns are classified on the basis of severity of liver damage as follows: Type 1, normal sera; Type 2, slight liver damage, *e.g.* chronic hepatitis; Type 3, moderate liver damage, *e.g.* acute hepatitis; Type 4, severe liver damage, *e.g.* cirrhosis.

3. Cases of infectious mononucleosis or malaria, which are often associated with liver damage, exhibit the same serum protein patterns, that is, Types 2, 3, and 4, as do cases of hepatitis or cirrhosis.

4. Serial studies on a single case of acute infectious hepatitis from the peak of the attack to the week after discharge show a continuous drop in the cholesterol unit value at 25° until a stationary level is reached and a gradual change in the pattern from Type 4 to Type 1 as the clinical condition of the patient improved.

5. The relationship of these studies to the electrophoretic studies of normal and liver disease sera is discussed.

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THE QUANTITATIVE DETERMINATION OF THE TWO TYPES OF BILIRUBIN SIMULTANEOUSLY PRESENT IN THE BLOOD, AND ITS CLINICAL IMPORTANCE

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Since the original publications of Hijmans van den Bergh[6] and his coworkers it has been known that the blood bilirubin can react in different ways with the diazo-reagent of Ehrlich. Hijmans van den Bergh stressed the fact that the difference between these 2 groups, the slow reacting and the quick reacting, was only relative and not absolute, being determined by the time necessary for the formation of the colored azo-bilirubin. With the quick or direct reacting bilirubin the formation of azo-bilirubin started immediately after the mixing of blood serum and diazo-reagent, and progressed quickly to maximum intensity. In contradistinction to this the slow or indirect reacting bilirubin gave no formation of azo-bilirubin after the mixing of blood serum and diazo-reagent, or this formation started only after several minutes and grew very slowly in intensity. When, however, a serum containing slow reacting bilirubin was treated with alcohol before mixing with the diazo-reagent, the coupling of the bilirubin and the reagent progressed quickly even with this form of bilirubin. As van den Bergh pointed out, there was no fundamental difference in reaction between the 2 kinds of bilirubin, the difference being only a relative one of time, which difference could be eliminated by using the device of alcohol-treatment. Van den Bergh considered the reactions in which a coloration due to azo-bilirubin became visible within 30 seconds as belonging to the direct type. This time was rather arbitrarily chosen and based on experience. From the start, however, it became clear that there were not 2 groups that could be sharply differentiated, but that many reactions were found in which the formation of the azo-bilirubin progressed in a somewhat different way. This led to the conception of the biphasic reaction, in which the formation of the azo-bilirubin started within 30 seconds, but did not progress quickly to its maximum as in the true direct reaction. The final color intensity was reached only very slowly in these reactions. The respective parts of these 2 phases in producing the ultimate color-intensity was different in most of these biphasic sera. In many of these reactions it was impossible to decide to which group the bilirubin in the serum belonged. As these biphasic reactions were frequent, this difficulty became one of the principal reasons why many investigators denied any clinical value to the van den Bergh reaction. These biphasic reactions, however, are probably the most interesting, and Hartog, one of the pupils of van den Bergh, has shown it to be probable that this peculiar behavior is due to the fact that 2 types of bilirubin occur simul-

taneously in the blood in amounts not differing greatly.

As long, however, as it was impossible to analyse the course of the reaction more precisely in regard to the time factor, and differentiation remained possible only on the basis of the occurrence of a coloration within 30 seconds or not, no further progress could be made. This has been changed nowadays by the photoelectric colorimeter, with which it is possible to make very exact determinations as to the time factor and the color-intensity due to the azo-bilirubin formation. This has led to the introduction of the 1 minute percentage, the percentage of the total blood bilirubin that is transformed to azo-bilirubin in 1 minute.

Extensive study of several hundred determinations of the reaction rate of bilirubin, together with the results of experiments have led us to the conclusion that this determination of the reaction rate supports the opinion that the 2 forms of bilirubin occur simultaneously in the blood, and that it is possible to deduce quantitatively the respective amounts of these 2 forms from the determination of reaction rate.

Technique. *a*. The total amount of bilirubin was determined by the method of Jendrassik and Cleghorn[4], in which the pH is brought to 6 by addition of sodium benzoate and caffeine. One ml. serum is mixed with 2 ml. caffeine reagent, 1 ml. water, and 1 ml. diazo-reagent.

Composition of Caffeine Reagent. Sol. *a*: sodium benzoate 30 g., caffeine 20 g., distilled water 70 ml. Sol. *b*: sodium acetate 58 g., distilled water 100 ml. Mixture of equal volumes of sol. *a* and sol. *b*.

Compositon of Diazo-Reagent. Diazo A: sulphanilic acid 1, hydrochloric acid 38% 15 ml., aq. ad. 1. L. Diazo B: solution of $NaNO_2$ 0.5%. Mixture of 10 ml. diazo A + 0.3 ml. diazo B.

The colorimeter (Lumetron 402-E) is adjusted to a standard solution of 1 ml. serum, 2 ml. caffeine reagent, 1 ml. water and 1 ml. diazo A (thus no formation of azo-bilirubin occurs in this mixture). A 1 cm. vessel and a filter M 515 are used. The light intensity is at its maximum. From the light extinction found, the percentage of bilirubin can be read with the help of a standard curve (see addendum). As the dilution is 5, the percentage found has to be multiplied by 10 to get the number of van den Bergh units.

b. The velocity of the azo-bilirubin formation was determined as follows: 1 ml. serum is diluted with 3 ml. 0.9% NaCl. To a reference solution of the same composition 1 ml. of diazo A is added and the colorimeter is adjusted to this reference solution. The intensity of the light is such that a change of 20 graduation marks is read. A 1 cm. vessel and a filter M 550 are used. For the determination of the rate of azo-bilirubin formation 1 ml. diazo-reagent is added to the diluted serum of the test sample and the percentage of transmission is read after 10 min. In this determination also the dilution is 5, so that the number of units (Hijmans van den Bergh) is found by multiplying by 10.

The use of different filters in Determinations *a* and *b* is necessary because the diazo reaction proceeds in an acid medium (pH 2 to 3) in the latter case, which gives a slightly different color from that in Determination *a*, which occurs at pH about 6. Experimentally it was shown that in both cases the same standard curve could be used with correct results (see *addendum*). To get reliable results it is important always to work under the same conditions. This absolute requirement is frequently neglected. *It is of paramount importance that the same dilution should be used in the determination of the total bilirubin content and the determination of the rate of azo-bilirubin formation.* When this method is used the time-difference in azo-bilirubin formation is demonstrated clearly for the various forms. In the direct variety the reaction starts immediately and proceeds quickly to its maximum intensity; in the indirect variety the diazotization progresses only slowly and gradually. In Fig. 1 an example is given of serum containing direct (quick) reacting bilirubin, obtained from a patient after an attack of cholelithiasis, and of serum containing indirect (slow) reacting bilirubin, obtained from a patient with hemolytic jaundice. The total amount of bilirubin in the 2 sera is nearly equal, showing that it is not the concentration of bilirubin which determines the type of reaction, but that a more fundamental difference between the bilirubins of these sera is concerned. Working with this method we came to prefer the 10

minute percentage readings to the determination of the 1 minute percentage. Inspection of the curves will clearly show why. The 1 minute reading is made when the quick reaction is in full progress, and an error of some seconds in the reading may make an important difference. When the reading is taken after 10 minutes, an inaccuracy of the same magnitude will make hardly any difference in result obtained, because the reaction has nearly come to a standstill at this time.

These 2 reactions demonstrate the most pronounced difference between the 2 possibilities with which bilirubin may react with the diazo-reagent. Between these 2 extremes every 10 minute percentage may be found in practice, comprising the so-called biphasic reactions. These 2 types of reaction curve are the exact and accurate registration of the 2 types of diazotization described by Hijmans van den Bergh.

What, however, is the significance of the intermediate rate of azo-bilirubin formation, part of which had already been recognized with the original and crude method as the puzzling biphasic reaction? Fundamentally there are 3 theories which try to explain the difference in rate of reaction:

1. The rate of reaction is dependent on the influence of the medium, *e.g.* the degree of dispersion of the serum colloids and many other factors.

2. The rate of reaction is dependent on the physico-chemical properties of only 1 variety of bilirubin in the blood. A different rate of azo-bilirubin formation can be explained by a different physico-chemical state of the bilirubin but the total amount of bilirubin in the body has at a given moment the same physico-chemical properties.

3. The ultimate velocity of the reaction is determined by the proportion in which a very quick reacting and a very slow reacting bilirubin occur simultaneously, as a mixture, in the blood.

We shall not discuss here whether the view is correct that the intrinsic difference between these 2 kinds of bilirubin exists in the difference that the slow reacting form is a bilirubin globin, whereas the quick reacting

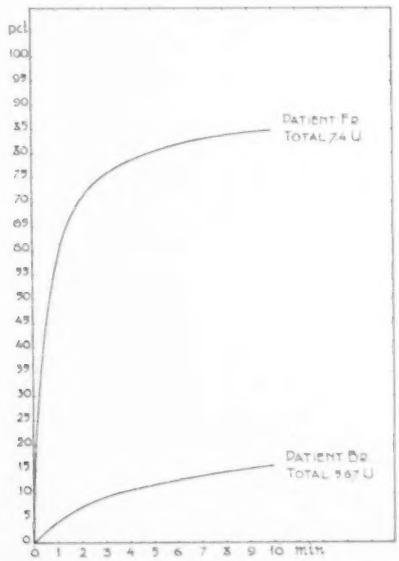

FIG. 1.—Bilirubin diazotization percentages in obstructive and hemolytic jaundice.

form is deprived of this protein fraction.

If we accept the assumption that there are 2 different forms of bilirubin, it must be taken as probable that mixtures of these 2 forms occur. Normally a certain amount of slow reacting bilirubin, derived from the hemoglobin of normal blood destruction, is present in the blood. When, as a result of an obstruction of the large bile ducts or damage to the liver parenchyma, quick reacting bilirubin enters the blood, the normal amount of slow reacting bilirubin will still be present and remain present, due to the blood-cell destruction continuing. It is even probable that in cases of parenchymal damage the slow reacting bilirubin is more slowly converted into the quick reacting form, as a result of which the slow reacting bilirubin is to a certain degree accumulated in the blood. The difference in solubility in chloroform, on the basis of which a separation of the respective amounts of the 2 bilirubins simultaneously present in the blood was attempted, underlines the probability that 2 different forms of bilirubin occur at the same moment[3,5,7]. Although with this method the separation of the 2 forms of bilirubin cannot be effected quantitatively in a reliable manner[2], a view that we can support on the basis of personal experience, the fact remains, that in this way the existence of 2 forms of bilirubin, differing in their solubility in chloroform, can be demonstrated. With this method it could in fact be shown to be probable that in many cases with quick reacting bilirubin there was also an accumulation of the slow reacting form.

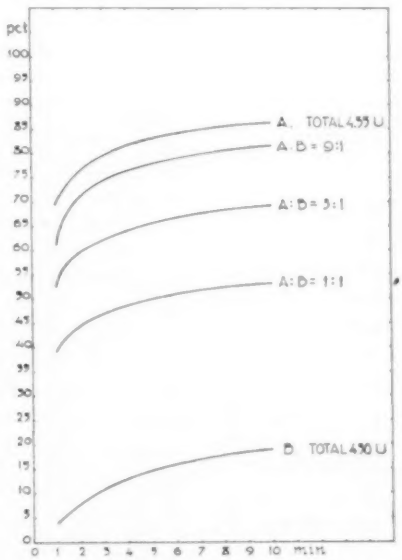

FIG. 2.—Reaction speeds of sera A and B and their various mixtures.

Neglecting the problem of the intrinsic physico-chemical difference between the 2 types of bilirubin, our experiments were carried out to investigate whether the differences in velocity of bilirubin diazotization were in accord with the conception of the existence of a bilirubin mixture or not. For this purpose mixtures were made of sera containing nearly equal

amounts of total bilirubin, the one quick reacting, the other slow reacting. The quick reacting serum A was taken from a patient after an attack of cholelithiasis. The slow reacting serum B came from a patient with hemolytic jaundice.

The various mixtures gave the following results:

Serum A total bilirubin 4.55 U., 10 min. percentage 86
Serum B total bilirubin 4.50 U., 10 min. percentage 19

Mixture	*10 min. percentage*
A:B = 9:1	82
A:B = 3:1	69
A:B = 1:1	54

The curves plotted from these reactions are given in Fig. 2. The curves obtained with the mixtures are absolutely comparable to those found in sera of patients with an intermediate rate of bilirubin diazotization. On the basis of a number of similar tests we drew up Table 1 from which the respective percentage amount of the 2 forms of bilirubin can be approximately derived from the 10 minute percentage found in a given sample.

Example: A serum contains a total bilirubin content of 5 U., 10 min. percentage 80. From Table 1 we see that 75% of the total amount consists of direct or quick reacting bilirubin, whereas 25% consists of the indirect or slow reacting form. The respective amounts are:

direct bilirubin $75/100 \times 5 = 3.75$ U.
indirect bilirubin $25/100 \times 5 = 1.25$ U.

In our opinion a rapid and easy determination of the respective amounts of the 2 bilirubins can be made in this way. This division Table was extensively checked with artificial mixtures. The following is an example, obtained with the mixture of a serum, Fr., obtained from a patient after an attack of cholelithiasis, and a serum, Br., from a patient with hemolytic jaundice (see Table 2).

A very good agreement is seen between the theoretically calculated amounts of bilirubin and the quantities calculated with the help of the Table from the experimentally found 10 minute percentage.

Although these results are already significant, it may be objected, that these experiments were performed with mixtures of 2 different samples of sera. There are certain reasons for believing that the mixing of sera might possibly influence the behavior of the bilirubin[1]. Therefore experiments were made in which the 2 different sera were not actually mixed, but in which 2 vessels of different size were used in one reading with the colorimeter. With ½ and 1 cm. vessels, filled alternatively with slow and quick reacting bilirubin, different combinations could be made. It was tested whether with these different combinations various 10 min. percentages would be found, thus making clear that different amounts of the 2 forms of bilirubin produce a related 10 min. percentage, and avoiding a possible influence of

TABLE 1.—PERCENTAGE OF THE 2 KINDS OF BILIRUBIN DERIVED FROM THE 10 MINUTE PERCENTAGE OF A GIVEN SAMPLE[2].

10 min percentage	*% direct (quick) bilirubin*	*% indirect (slow) bilirubin*
30	20	80
40	25	75
50	37.5	62.5
60	50	50
70	62.5	37.5
80	75	25
90	80	20

real mixing of the 2 sera. In the first combination a serum Br. (familial hemolytic jaundice) and a serum H (toxic hepatitis in pneumonia) was used. Theoretically the different amounts of the 2 types of bilirubin, present in the 2 vessels, could be calculated with the help of Table 1. Then the 2 vessels were placed in the colorimeter and the 10 min. percentage was determined. From this percentage, experimentally found, again with the help of the above Table the amounts of the 2 types of bilirubin were calculated. From the figures given it can be seen that the agreement is very close (see Table 3).

A second and similar combination was made with serum v.d.R. (constitu-

TABLE 2.—RESULTS FROM MIXTURES OF 2 TYPES OF SERA

Sera	*Total Bilirubin*	*10 min. %*	*Direct Bilirubin*	*Indirect Bilirubin*
Fr.	7.4 U.	85.1	5.74 U.	1.66 U.
Br.	5.67 U.	16	0.0 U.	5.67 U.

MIXTURES.

	Theoret. calculated amount			*Experimentally found*		*Amount Calculated from this with Table 1*	
Ratio	*Total*	*Direct*	*Indirect*	*10 min. %*	*Total*	*Direct*	*Indirect*
Fr : Br = 3 : 1	6.97	4.31	2.66	68.1	7.34	4.40	2.94
Fr : Br = 1 : 1	6.53	2.87	3.66	54.7	6.63	2.90	3.73
Fr : Br = 1 : 3	6.10	1.44	4.66	36.7	6.30	1.48	4.82

TABLE 3.—THE 2 TYPES TEST SEPARATELY AND COMBINED

Sera	*Total Bilirubin*	*10 min. %*	*Direct Bilirubin*	*Indirect Bilirubin*
Br	1.15 U.	5.5	—	1.15 U.
H	3.3 U.	82	2.5 U.	0.8 U.

COMBINATION TEST.

		Theoret. calculated amount			*Experimentally found*	*Amount calculated from this with table*	
Vessel		*Total*	*Direct*	*Indirect*	*10 min. %*	*Direct*	*Indirect*
Br	H						
½ cm	½ cm	2.23	1.25	0.98	68	1.33	0.90
½ cm	1 cm	2.59	1.67	0.92	61.5	1.59	1
1 cm	½ cm	1.87	0.83	1.04	60.5	0.94	0.93

TABLE 4.—THE SAME TEST AS IN TABLE 3 WITH A DIFFERENT PAIR OF SERA

Sera	*Total Bilirubin*	*10 min. %*	*Direct Bilirubin*	*Indirect Bilirubin*
v.d.R.	5.1 U.	17.2	—	5.1 U.
H	1.78 U.	76.7	1.26 U.	0.52 U.

COMBINATION TEST.

		Theoret. calculated amount			*Experimentally found*	*Amount calculated from this with table*	
Vessel		*Total*	*Direct*	*Indirect*	*10 min. %*	*Direct*	*Indirect*
v.d.R.	H						
½ cm	½ cm	3.44	0.63	2.81	33.4	0.75	2.69
1 cm	½ cm	3.99	0.42	3.57	22.7	±0.39	±3.60
½ cm	1 cm	2.89	0.84	2.05	35.7	0.66	2.23

tional hyperbilirubinemia) and serum H (toxic nepatitis in pneumonia). The results were as shown in Table 4.

These results gave support to the opinion that the division Table can be used to determine the relative amounts of the 2 forms of bilirubin from the 10 min. percentage.

The necessity for this differentiation had been felt for a long time, but on the basis of the chloroform solubility this could only be done by means of a rather laborious and complicated procedure the reliability of which could justly be doubted. The result was that this method was not accepted as a routine clinical test. On the basis of these experiments it seems justifiable to make this differentiation with the help of the already generally accepted determination of the rate of bilirubin diazotization. This differentiation gives a better insight into the composition of the total bilirubin of the blood than does the determination of the 1 min. or 10 min. percentage alone. When a total amount of 2 U. with 10 min. percentage 60 is found, the situation is totally different from that existing when a total of 9 U. with 10 min. percentage 60 is found. In the first case we find (calculating with the help of the Table) that 1 U. of direct bilirubin and 1 U. of indirect bilirubin are present in the blood, whereas in the latter case 4.5 U. of direct and 4.5 U. of indirect bilirubin are present. Thus in the first case no indirect bilirubin has accumulated in the blood, indicating that one of the most fundamental functions of the liver—the conversion of the bilirubin of indirect type into that of the direct type—is intact, whereas in the latter case the accumulation of indirect bilirubin indicates that this fundamental function of the liver is deficient.

Comment. It has been shown experimentally to be probable that the rate of bilirubin diazotization, expressed in the 10 min. percentage, (*i.e.*, the percentage of the total bilirubin of the blood that is converted into azobilirubin after 10 minutes) is determined by the relative amounts of 2 different types of bilirubin, a slow reacting and a quick reacting form, simultaneously present in the blood. On the basis of the value found for the 10 min. percentage the respective amounts of these 2 forms of bilirubin can be derived from the total amount of bilirubin in the blood. This gives a more differentiated insight into the process that is going on in the liver. When the conversion from indirect bilirubin into direct bilirubin is regarded as a fundamental function of the liver parenchyma, the accumulation of indirectly reacting bilirubin indicates that this function has been damaged. The determination of this amount of indirect bilirubin was not possible hitherto by a generally accepted method, because the share of the indirect bilirubin in the color formation was blurred by the reaction of the direct bilirubin. The thesis can be defended that the presence of direct bilirubin is the expression of the leakage of the already converted bilirubin out of the system of bile capillaries into the blood stream, whereas the amount of indirect bilirubin indicates the functional capacity of the liver parenchyma itself. Thus the determination of these various compounds should give an insight into the anatomical and functional restoration of the liver. Because this simple method makes it possible to determine the respective amounts in clinical routine work, the possibility is offered of investigating whether this differentiation gives a better insight into the anatomical and functional condition of the diseased liver.

A few illustrative examples can be given. These examples are representative of their groups of liver diseases

with jaundice and give the results as they are commonly found in practice in these diseases.

Clinical Examples. 1. TRANSITORY JAUNDICE AFTER AN ATTACK OF CHOLELITHIASIS. Total bilirubin 7.4 U. 10 min. percentage 85.1 (Direct, 5.74 U. Indirect, 1.66 U). In this case there is hardly any damage to the function of the liver cells as far as the conversion from indirect into direct bilirubin is concerned. This can be deduced from the normal or hardly elevated indirect bilirubin in the blood. The temporary obstruction had given rise to rupture of the closed system of the bile capillaries. The ampulla is assumed to be the anatomical location of this rupture.

As the obstruction is presumed to be of short duration in such a case, the direct bilirubin is further excreted by the bile ducts (Fig. 3).

2. LIVER DAMAGE AFTER OBSTRUCTION OF LONG STANDING. It is well known that in such cases a certain amount of damage occurs to the liver parenchyma. An illustrative case of this kind follows.

CASE B1. Complete obstruction of the large bile duct by common-duct stone of 2 months' duration (diagnosis confirmed at operation). Total bilirubin 23.48 U. 10 min. percentage 76.5 (Direct, 16.77 U; Indirect 6.71 U).

The elevation of the indirect fraction of bilirubin indicates the damage of the liver parenchyma, partly preventing conversion of the indirect form of bilirubin into the direct form of bilirubin. The direct fraction is present in the blood because of the rupture of the bile capillary system after this long obstruction. After an obstruction of long duration the rupture is not only located in the ampulla, but the intercellular bile capillaries also are distended and ruptured (Fig. 4).

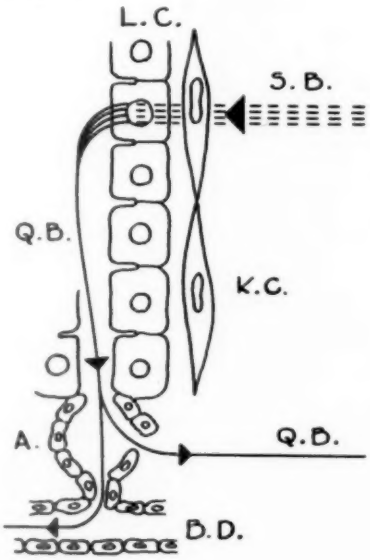

FIG. 3

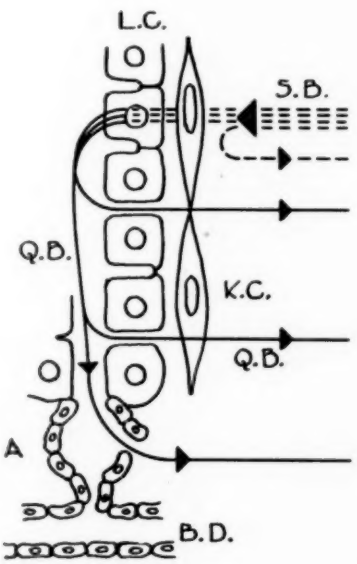

FIG. 4

FIG. 3. Diagram of Results of temporary obstruction of large bile ducts, without distention of the intercellular bile capillaries or functional or anatomical disturbance of the liver parenchyma. The slow-reacting bilirubin (S.B.) is well converted into quick-reacting bilirubin (Q.B.). Part of the latter re-enters the blood stream through the disrupted ampulla. Result: normal amount of slow-reacting bilirubin, varying amount of quick-reacting bilirubin in the blood.

FIG. 4. Prolonged obstruction of large bile ducts, with distention and rupture of the intercellular capillaries and slight functional disturbance of the liver parenchyma. Due to this, part of the slow-reacting bilirubin is not converted into quick-reacting bilirubin, while part of the direct bilirubin re-enters the blood stream through the disrupted bile capillaries and ampulla. Result: slightly elevated slow-reacting bilirubin and a varying amount of quick-reacting bilirubin in the blood.

3. Jaundice is Due to Parenchymal Damage with Open Bile Ducts. Case de K. Subacute necrosis of the liver in pregnancy. Total bilirubin 29.2 U. 10 min. percentage 72 (Direct, 18.98 U; Indirect 10.22 U.). Only part of the indirect bilirubin is converted into the direct form, so that there is accumulation of indirect bilirubin in the blood. As the continuity of the bile capillary system is broken because of the damage to the liver cells, the direct bilirubin can leak out of the bile duct system and re-enter the blood stream (Fig. 5).

4. Constitutional Hyperbilirubinemia. Here it is assumed that the liver cells have a lowered capacity to convert indirect bilirubin into the direct form. Thus a certain amount of indirect bilirubin is retained in the blood. As the liver does not show anatomical changes the direct bilirubin comes into a closed capillary system, so there is no leakage back from the capillaries into the blood stream (Fig. 6).

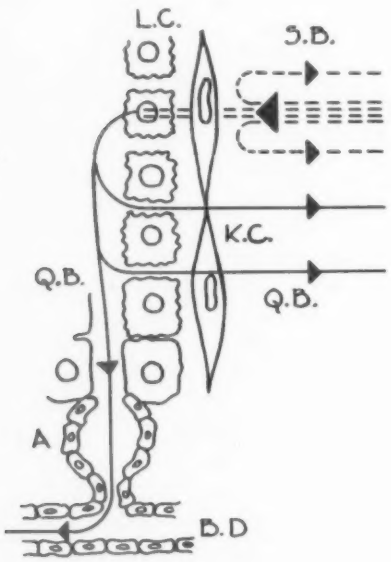

Fig. 5

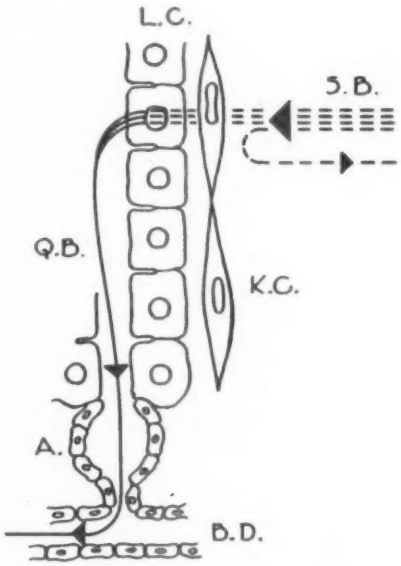

Fig. 6

Fig. 5. Damage to liver parenchyma with functional disturbance and breaking of the continuity of the bile capillary system. Only part of the slow-reacting bilirubin is converted into the quick-reacting form, while part of this latter re-enters the blood stream. Result: accumulation of slow-reacting bilirubin in varying amounts, and presence of varying amounts of quick-reacting bilirubin in the blood.

Fig. 6. Functional disturbance of the liver parenchyma without anatomical damage. Part of the slow-reacting bilirubin is not converted into the quick-reacting form, thus slight accumulation of slow-reacting bilirubin occurs in the blood. Constitutional hyperbilirubinemia might be an example of this type. An elevated total bilirubin with low 10 min. percentage is found. Although the exact place where the conversion from the slow-reacting into the quick-reacting form takes place is not known—Kupffer cell or liver cell—for the sake of clearness the drawings are made on the assumption that this happens in the liver cells.

The Figures are a combination of those given by Rich, A. R.: Bull. Johns Hop. Hosp., **47**, 338, 1930, and Watson, C. J.: Blood I, 103, 1946

Abbreviations for Figs. 3 to 6: S.B. = slow-reacting bilirubin
Q.B. = quick-reacting bilirubin
L.C. = liver cell
K.C. = Kupffer cell
A. = ampulla
B.D. = bile duct

Technical addendum[4a]. DETERMINATION OF STANDARD CURVE. 10 mg. of pure bilirubin were dissolved in 100 ml. chloroform. 5 ml. of this solution were diluted with absolute alcohol to 50 ml. From this standard solution dilutions were made with absolute alcohol, corresponding to 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg. bilirubin per 100 ml., after which 1 ml. diazo-reagent (10 ml. diazo-reagent A + 0.3 ml. diazo-reagent B) were added. From the color scale, obtained in this way, the percentages of light transmission were determined, using a 1 cm. vessel and filter M 550. The figures obtained, when plotted on semi-logarithmic paper, gave an approximately straight line. To facilitate

TABLE 5.—STANDARD CURVE RELATION BETWEEN % TRANSMISSION AND MG. % BILIRUBIN.

% Transmission	*mg. % bilirubin*	*% Transmission*	*mg. % bilirubin*	*% Transmission*	*mg. % bilirubin*
100	0.000	74	0.178	48	0.393
99	0.001	73	0.185	47	0.403
98	0.002	72	0.192	46	0.414
97	0.004	71	0.200	45	0.425
96	0.006	70	0.207	44	0.436
95	0.010	69	0.214	43	0.448
94	0.014	68	0.221	42	0.460
93	0.020	67	0.228	41	0.472
92	0.030	66	0.235	40	0.484
91	0.042	65	0.242	39	0.497
90	0.054	64	0.250	38	0.510
89	0.066	63	0.258	37	0.523
88	0.079	62	0.266	36	0.536
87	0.090	61	0.274	35	0.550
86	0.100	60	0.282	34	0.565
85	0.106	59	0.290	33	0.580
84	0.112	58	0.299	32	0.596
83	0.118	57	0.308	31	0.612
82	0.124	56	0.317	30	0.628
81	0.130	55	0.326	29	0.645
80	0.136	54	0.335	28	0.663
79	0.143	53	0.344	27	0.681
78	0.150	52	0.353	26	0.700
77	0.157	51	0.363	25	0.720
76	0.164	50	0.373	24	0.740
75	0.171	49	0.383	23	0.762
				22	0.785
				21	0.808

The tint of azo-bilirubin at pH 2 to 3 is slightly different from that at pH6: in the latter maximum extinction is obtained with filter M 515. Table 3 shows that with both filters M 550 and M 515 the same standard curve can be used without appreciable error. (The solutions of bilirubin were brought to the desired pH with a few drops of caffeine reagent.)

TABLE 6.—RELATION BETWEEN % TRANSMISSION AND MG. % BILIRUBIN USING DIFFERENT FILTERS AT pH2 AND pH6.

	% Transmission	
mg. bilirubin per 100 ml.	*pH2 filter M 550*	*pH6 filter M 515*
0.1	86	86.5
0.2	71	72.5
0.3	58	59
0.4	47.3	47
0.5	39.2	39.2
0.6	31.2	30

reading of values lower than 0.1 mg. per 100 ml. with sufficient accuracy, a separate curve was made with the help of a 5 cm. vessel. In this way Table 2 was drawn up.

Summary. A method is described, based on experimental data, by which the various amounts of direct (quick) and indirect (slow) reacting bilirubin simultaneously present in the blood can be determined. This separation is based on determination of the rate of azo-bilirubin formation, already current in clinical work. The determination of these various fractions will probably give an insight into the anatomical and functional state of the liver parenchyma.

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DISTRIBUTION OF PENICILLIN IN THE BODY BY VARIOUS TREATMENT METHODS

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In a previous study[7] it has been demonstrated that the chemotherapeutic effectiveness of penicillin does not necessarily parallel the blood level curves. From the experience of other authors[2,3], it would appear that therapeutic effectiveness changes with the method of therapy to an extent which could not be foreseen from the blood concentrations alone. One of the factors responsible for these observations might be the accumulation and distribution of penicillin in the organs. The urinary elimination of penicillin[15,18] and its presence in the lymph[21] at negative blood levels is quite suggestive of such an interpretation. Though the distribution of penicillin in the tissues following low doses or at short intervals after administration has been studied[5,11,17], the information available on its fate following repository treatment, or when penicillin blood levels are no longer demonstrable, is insufficient. The present study represents an analysis of the relationship between the fate of the antibiotic in the host organism and the therapeutic effectiveness. During the course of this investigation further information has been obtained on the distribution of penicillin in body fluids and organs, particularly in relation to dosage, vehicle, and blood concentrations.

Materials and Methods. Penicillin Assay. Three methods of penicillin assay were employed: (a) The serial dilution method with hemolytic Streptococcus S 4 as test organism and defibrinated rabbit blood as indicator of the hemolysis endpoint, (b) The serial dilution method suggested by Welch and coworkers[16], using *Bacillus subtilis* 9675 as test organism, (c) The cup-plate method with *Staphylococcus aureus* 209 as test organism. The results of dilution tests were verified by subcultures of all tubes on blood agar plates; the sensitivity of this method varied between 0.015-0.03 units per ml.

Preparations. Crystalline potassium penicillin G (potency 1595 u. per mg.) was used throughout the study. The repository penicillin preparation consisted of suspensions of this salt with 1.0 mg. per ml. of epinephrine in peanut oil; 0.1 ml. per 100 gm. rat was injected intramuscularly in the gluteus muscle. Occasionally, as indicated in the text, commercial suspensions of procaine penicillin in oil were used.

Samples. Blood was obtained from the axillary artery of anesthetized rats (100 to 200 gm. body weight) and from the femoral vein of dogs (average weight 10 kg.). The blood was allowed to clot and the serum obtained by centrifugation. Cerebrospinal fluid was drawn from dogs—which were in most cases under (barbiturate) anesthesia—by inserting a 22 gauge needle into the cisterna magna and allowing the fluid to drop into sterile tubes. The tissues were removed aseptically and mashes were prepared by mechanical grinding, as previously described[19].

For determination of the locally retained antibiotic, the injected gluteus muscle of rats was dissected and cut slices were placed on assay plates. The inhibition zone provided,

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for our comparative purposes, a satisfactory measurement of the penicillin present in the muscle tissue. All samples were kept at 5° C. and assayed on the day of collection. Single experiments were carried out on groups of 15 to 50 rats; organ samples of 2 to 4 animals were occasionally pooled. The results of about 70 experiments carried out over a period of 2 years are considered in this paper.

Bacterial Infection. As in the preceding studies, 1.0 ml. of an 18-hour culture of Pneumococcus Type I was inoculated intraperitoneally into 100 to 150 gm. rats *previously* treated with penicillin. The inocula, corresponding to 10 to 100 lethal doses, varied from dilutions of 10^{-5} to 10^{-6}. Rats surviving a 3 week period were adjudged cured. The value of this classical method for determining the duration of therapeutic effectiveness has also been confirmed recently in the case of penicillin treatment by A. Katherine Miller and co-workers[14].

Results. (1) Chemotherapeutic Effect/Blood Level Duration Relationship, and Vehicle. As a part of this problem, we investigated whether there are differences in the *therapeutic activity per blood level duration* relationship using different vehicles. For the repository injection with penicillin in oil + vasoconstrictor, it already has been established[1] that the prophylactic action against Pneumococcus Type I infection outlasts by about 6 hours the detectable blood level (20 hrs.). In similar experiments with aqueous intramuscular penicillin treatment, durations of blood level and therapeutic effectiveness were compared. According to the data of Table I, even with *high aqueous doses* (10,000 u. per 100 gm. and over), the duration of the chemotherapeutic effectiveness does not exceed the limits of the blood level (4 hrs.).

(2) Blood and Organ Levels by Different Routes. The distribution of the antibiotic in the various organs of rats (blood, kidney, lung, liver, spleen), following the intramuscular, subcutaneous and oral administration of various aqueous penicillin dosages, was determined. In the course of this investigation we noted[12] that the liver suspensions of untreated rats gave a specific inhibition of hemolytic Streptococcus. Following this observation it was necessary to reevaluate a number of liver figures and to control the organ determinations in which we were interested by using *B. subtilis* as test organism. On the other hand, we found that *B. subtilis* is greatly inhibited by rat and rabbit serum—and somewhat less by dog serum—even after inactivation at 56° C. for half an hour. Thus, in order to have reliable results, Streptococcus had to be used for the serum and *B. subtilis* for the organ assays. The results established in this way on a significant number of rats (177) at the same *high dose level*—5000 u. per 100 gm.—indicate that (a) there is no difference in blood level duration following oral, subcutaneous, and intramuscular routes (Fig. 1); (b) the liver, spleen, and lung levels are shorter than the blood levels; (c) the kidney levels, on the contrary, invariably last longer than the blood levels; (d) the oral and subcutaneous routes give kidney levels of the same duration, while the kidney level is perhaps slightly longer after intramuscular administration. The relative distribution of the antibiotic among the various organs of rats is in agreement with the results of Richardson and his co-workers[17] who studied the continuous intravenous injection on dogs. The shorter duration and lower concentration of the penicillin in the lung, as compared to the blood, corresponds to the findings of Laurent, McIlroy and Hadley[11]. Further, we found that by increasing the aqueous penicillin dose, the duration of the kidney level is increased more than that of the blood level. For instance, by increasing the 5000 u. per 100 gm. dose to 20,000 u. per 100 gm. (by the oral and subcutaneous routes), the duration of the blood level increases from 2½ to only 3

hours, while the kidney level increases from 4½ to 5 to 9 to 11 hours. *This finding suggests that improved chemotherapeutic activity might follow larger penicillin doses, due to the longer presence of the antibiotic in the organism, even if the blood level itself reveals no advantage over the lower dosage form.*

(3) Influence of the Vehicle on Blood and Organ Levels. We studied the distribution of the antibiotic in the various organs comparing the effect of aqueous and repository treatments. Fig. 2 shows the influence of the vehicle on the distribution of penicillin in the organs with the 5000 u. per 100 gm. dose. By the repository injection, the duration of detectable penicillin in the lungs, spleen, and liver is shorter, and the concentration is lower, than in the blood; only in the kidney and in the urine is the duration longer. With the same dose, aqueous penicillin gave blood levels of 3 to 4, and kidney levels of about 8 hours duration, whereas repository penicillin gave blood levels of 12, and kidney levels of as much as 24 hours duration. Higher doses of repository penicillin, such as 10,000 and 20,000 u. per 100 gm., gave blood levels of 14 and 16 hours, and kidney levels of 30 and even 40 hours duration. It would

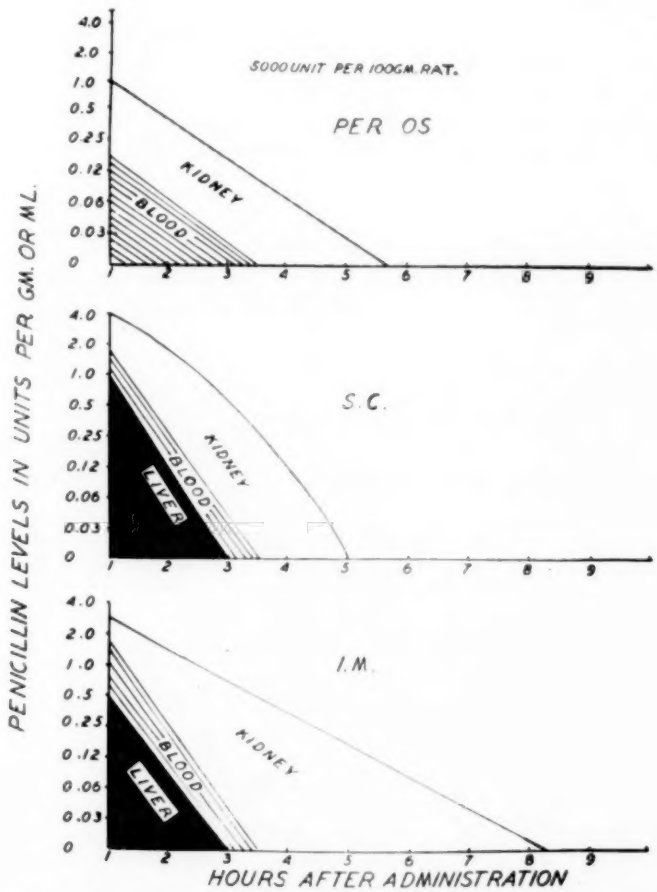

Fig. 1. Duration of penicillin in blood and organs following large aqueous doses.

seem from these data that the kidney level responds more definitely to dosage differences than the blood level, particularly with repository penicillin.

(4) Cerebrospinal Fluid Levels. The intramuscular injection of 100,000 and 200,000 u. aqueous penicillin in 6 dogs of about 10 kg. body weight gave, in 3 cases only, barely detectable CSF levels of short duration, in spite of the high blood levels obtained in all dogs (4 to 20 u. per ml.). By increasing the intramuscular aqueous dose to 300,000 and 400,000 u., 8 out of 10 dogs showed CSF levels which lasted for 2 to 4 hours. All 12 dogs injected intravenously with 300,000 u. demonstrated detectable levels, which generally ended 3 to 4 hours after injection. It seems that the appearance of the penicillin in the CSF takes place earlier after intravenous (less than half an hour) than following intramuscular (1 to 2 hours) injection (Fig. 3). Two doses of 300,000 u. Procaine penicillin, injected 10 hours apart, resulted in a constant CSF level of 0.03-0.06 u. per cc. during the 7 hour observation period following the second injection. (We are not in a position to make any statement on the influence which the anesthesia or the method of collection of the samples might have had on the penetration of the antibiotic into the CSF, since our comparative data from this standpoint are not sufficiently extensive.)

The trend of our experiments corresponds closely to the early clinical data of McDermott and Nelson[13], who showed that only high aqueous doses, such as 300,000 to 500,000 u. give positive spinal fluid levels, though Cooke and Goldring[4] reported high CSF concentration in 4 out of 9 children treated with 500 to 5000 u. per kg. More recent

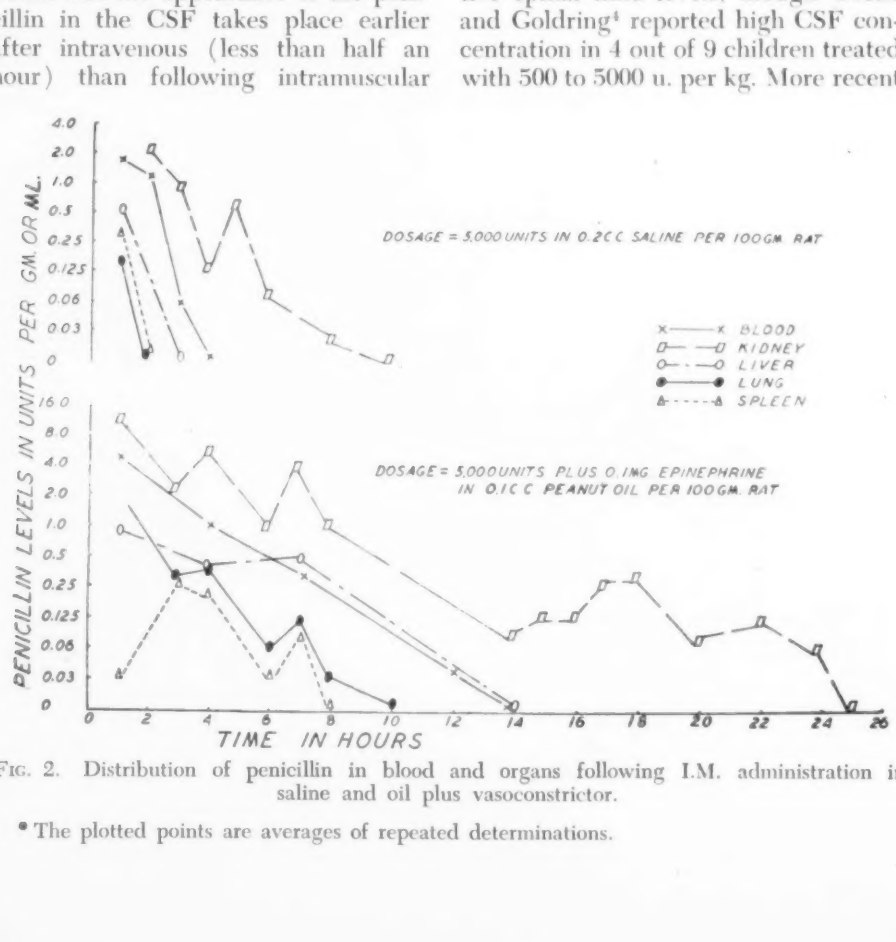

Fig. 2. Distribution of penicillin in blood and organs following I.M. administration in saline and oil plus vasoconstrictor.

* The plotted points are averages of repeated determinations.

data obtained on humans, and these experiments,* indicate very convincingly that the diffusion in the CSF requires prolonged and very high plasma levels. These have been obtained by Boger, Baker and Wilson[1] by intermittent intramuscular aqueous injections of 100,000 u. and by Janowitz *et al.*[10] by repeated intravenous administration of 1,000,000 u. Both groups reported higher CSF concentrations with the use of caronamide, which increases the penicillin plasma levels. Schwemlein, Barton, Bauer, Loewe, Bundesen and Craig[20] found that by continuous intravenous therapy a total dosage of 10 and 20 million u. is required to induce detectable CSF levels in 71% and 100% of the patients, respectively. It can be concluded that in order to maintain the antibiotic in the CSF, extremely high doses far above those required to induce high blood levels should be administered parenterally. If we could translate our experiments on dogs to clinical practice, a single minimal dose of about 2,000,000 u. of aqueous parenteral penicillin would be required to induce detectable CSF levels.

(5) Repeated Aqueous vs. Single Repository Treatment. What are the conditions responsible for these 2 effects noted for repository penicillin: the marked prolongation (beyond blood levels) of the kidney level, and of the chemotherapeutic effectiveness? Do these effects depend on the continuous maintenance of the penicillin in the blood stream only, or rather on some other factor related to the vehicle?

To answer this question, repeated doses of aqueous penicillin resulting in continuous blood levels were given to rats in a number of experiments. The doses ranged from 300 to 5000 u. per 100 gm. and were repeated up to 10 times, at intervals of 1 to 4 hours. Fig.

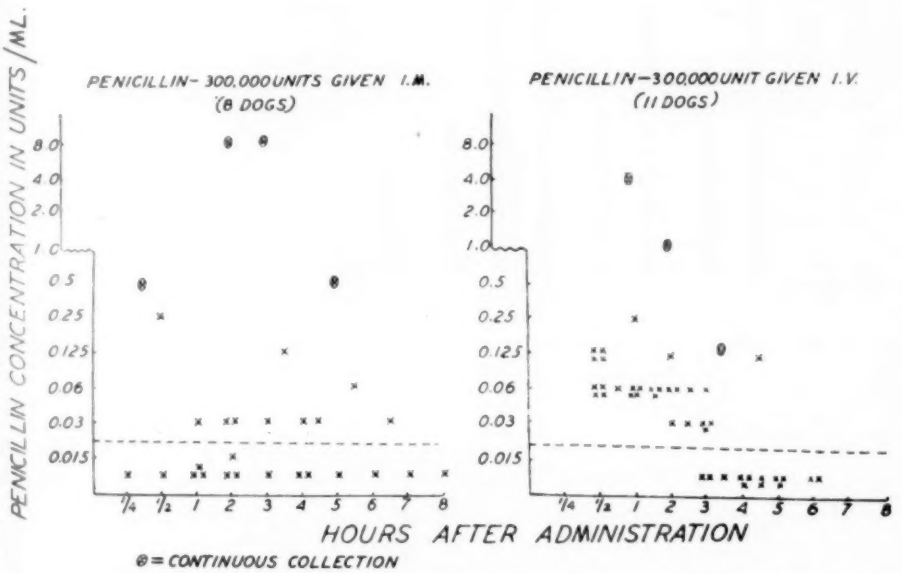

Fig. 3. Penicillin concentrations in cerebrospinal fluid following single aqueous doses in dogs.

* A preliminary report has been presented at the 48th General Meeting of the Soc. of Am. Bacteriologists in Minneapolis, May 1948. Proc. of Meeting Soc. Am. Bact., 1, 75, 1948.

4 illustrates a typical experiment. The blood and kidney levels following the 10th hourly, intramuscular injection of 1000 u. aqueous penicillin do not differ significantly from those obtained after the first injection. Thus, by maintaining continuous blood levels with repository and repeated aqueous penicillin concentration in the lungs of 12 rats, sacrificed 5 hours after the last injection. Similarly, 7 treatments with 5000 u. per gm. at 3 hour intervals gave an average of 0.125 u. per gm. in the lungs of 4 rats sacrificed 3 hours after the last injection, when the blood levels were already negative. Characteristical-

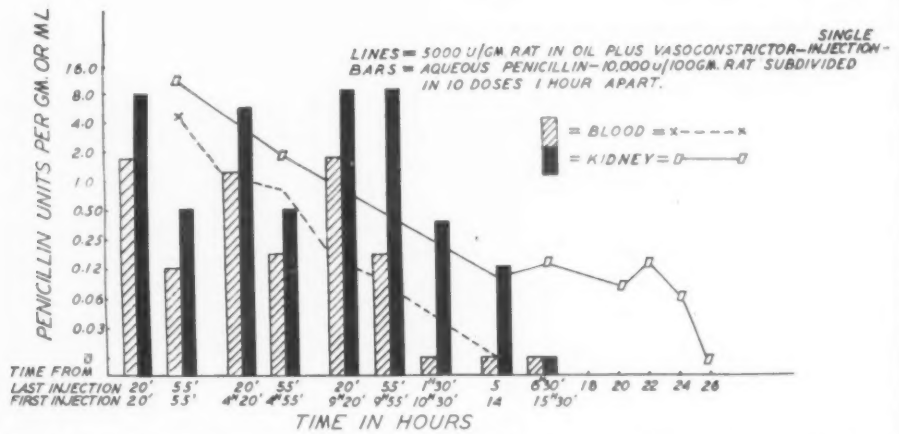
LINES = 5000 U/GM. RAT IN OIL PLUS VASOCONSTRICTOR—INJECTION—SINGLE
BARS = AQUEOUS PENICILLIN—10,000 U/100 GM. RAT SUBDIVIDED IN 10 DOSES 1 HOUR APART.
= BLOOD = x- - - - x
= KIDNEY = o——o
PENICILLIN UNITS PER GM. OR ML
16.0
8.0
4.0
2.0
1.0
0.50
0.25
0.12
0.06
0.03
0
TIME FROM LAST INJECTION 20' 55' 20' 55' 20' 55' 1H30' 5 6H30' 18 20 22 24 26
FIRST INJECTION 20' 55' 4H20' 4H55' 9H20' 9H55' 10H30' 14 15H30'
TIME IN HOURS

FIG. 4. Comparison of penicillin blood and tissue levels in rats following repeated aqueous and single repository intramuscular treatment.

TABLE 1.—DURATION OF CHEMOTHERAPEUTIC EFFECT OF AQUEOUS PENICILLIN AGAINST PNEUMOCOCCUS I INFECTION OF RATS.

I. M. dose u/100 gm.	*Infection after treatment* (Hours)	*% rats positive blood level*	*Duration of negative blood levels* (Hours)	*No. rats*	*% survived (days)* 8	15	21
10,000	4	12	0	20	35	30	*
	4½	0	½	10	10	10	10
	6	0	2	20	25	0	0
30,000	6	15	0	10	60	20	20
Controls	–	—	–	70	17	12	10

* Killed @ 15 days—all heart and organ cultures negative including those of 2 surviving controls.

treatment the retention of penicillin in the organism is of different durations.

It is worth while to report that in some cases we found penicillin retention in the lungs of animals treated repeatedly with extremely high aqueous doses. For instance, 4 times 5000 and 10,000 u. given at 4 hour intervals, produced an average of 0.3 u. per gm. ly, the kidney level in this experiment ended 7 hours after the last injection.

(6) ABSORPTION OF PENICILLIN FROM THE SITE OF INJECTION. Our findings, showing that the effect of the treatment as far as therapeutic utilization, accumulation, and organ levels is concerned is different with various vehicles, led us to investigate the condi-

tions of local absorption. It was found that an oily vehicle induces a longer retention of the penicillin at the site of the injection than an aqueous vehicle, though the differences in blood level duration are negligible between these two forms of administration. For instance, in the muscles injected with 10,000 u. per 100 gm. in oil, high bacteriostatic effect was recorded 16 hours after treatment, while no antibiotic action was noticed 10 hours after aqueous injection of the same dose, though the blood levels were approximately the same with the 2 vehicles. The same amount of penicillin injected in the form of a repository mixture, in oil + vasoconstrictor, revealed the presence of antibiotic in the muscle 20 and 24 hours after injection, though the blood level was already negative 16 hours after treatment. These experiments (Table 2) prove that with oily vehicles there is penicillin at the site of injection long after the disappearance of detectable amounts of the antibiotic from the blood stream. It is considered likely that the prolongation of the presence of penicillin in the organism at concentrations below detection in the blood, as revealed by the kidney levels, depends on the continuous absorption of this residual deposit. It certainly is not coincidental that there is a relationship between duration of kidney levels and locally present penicillin following oily or repository treatment. Further evidence of this supposition might be found in the observation that *very high* aqueous penicillin doses

TABLE 2.—LOCAL ABSORPTION OF PENICILLIN AND BLOOD LEVELS FOLLOWING INTRAMUSCULAR ADMINISTRATION IN VARIOUS VEHICLES.

Hours after treatment	*500 u/100 gm.*						*2000 u/100 gm.*					
	saline			*oil*			*saline*			*oil*		
	m	*b*		*m*	*b*		*m*	*b*		*m*	*b*	
½	24.4	1.3	(3)	39.0>2.0		(2)	35.2>2.0		(2)	34.5>2.0		(2)
¾	26.0	0.37	(2)	32.2	0.5	(2)	22.0	0.37	(2)	33.2	2.0	(2)
1	0	0.06	(1)	20.0	0.06	(1)	19.8	0.31	(3)	30.8	1.5	(3)
1½							21.0	0.06	(2)	23.0	0.37	(2)
2	0	0	(1)	18.0	0	(1)	0	0	(1)	39.0	0.25	(1)
4	0	0	(1)	28.0	0	(1)	0	0	(1)	25.0	0	(1)
6							0	0	(1)	28.0	0	(1)

Hours after treatment	*10,000 u/100 gm.*						*20,000 u/100 gm.*					
	saline			*oil*			*oil + epineph.*			*saline*		
	m	*b*		*m*	*b*		*m*	*b*		*m*	*b*	
½												
¾												
1												
1½												
2										61.0>2.0		(2)
4										47.0	0.12	(2)
6				42.5	0	(2)				39.0	0.09	(2)
8–10	0	0	(2)	22.5	0	(4)	51.5	1.0	(2)	28.3	0	(4)
14	0		(4)	12.5		(2)	42.8	0.06	(4)			
16				12.5		(2)	43.2	0	(4)			
18–24							22.5	0	(14)			

Note: m = mm inhibition of *Staphylococcus aureus* by muscle slice
b = penicillin u/cc. serum (Rammelkamp)
Figure in parentheses indicates number of experiments averaged
Epineph. = Epinephrine

(200,000 u. per kg.) induce longer penicillin retention at the site of injection and more prolonged kidney levels, although no significant increase in the blood level duration is noticeable.

It is quite possible that, besides the local retention, a certain saturation of the tubular excretory mechanism might contribute to this increase of the kidney level. Such a saturation has been reported by Eagle and Newman[6] in giving higher doses, approximately 1,000,000 u. per kg. (= 600 mg. per kg.), intramuscularly to rabbits.

Discussion. An interesting conclusion to be drawn from these experiments is that the therapeutic utilization of penicillin might be different, *even at equality of blood level durations*, by using different vehicles. The prophylactic experiments reveal that even in relation to the detectable blood level duration, repository penicillin treatment gives a more lasting effect than the aqueous treatment. Undoubtedly, this more prolonged effectiveness of the repository treatment depends on the longer retention in the organism of the penicillin, which although below detection in the blood, can be demonstrated by positive kidney levels. There is no direct quantitative relationship between chemotherapeutic effectiveness and height of kidney concentration at the periods of *negative* blood level. However, if the length of the duration of the kidney levels is considered, such a relationship is established by this and the previous study[1]: after the disappearance of detectable penicillin from the blood, a duration of at least 10 hours of *detectable kidney level* is required to obtain protection against Pneumococcus I infection with 10 to 100 lethal doses. Since, with high aqueous doses, prolongation of the kidney level beyond the blood level reaches maximal time periods of 2 to 5 hours only, this protective effect cannot be obtained if the animal is infected toward the end of the aqueous blood level curve. One must admit the possibility that there might be a further phase in the "undetectable period," when the penicillin though present is no longer detectable bacteriologically in any of the organs. However, this assumption should not influence the relative values obtained for the various methods of administration.

The experimental analysis of the conditions responsible for the greater retention and higher utilization of repository penicillin leads to the conclusion that these effects do not depend on the length of the period of continuously sustained blood levels. In fact, treatments even with very high aqueous doses (10,000-50,000 u. per kg.) at close intervals (1 to 2 hours), resulting in high blood levels for the whole treatment period, did not duplicate the results of the single repository injection.

The following interpretation accounts for the majority of these findings. Aqueous penicillin is rapidly absorbed from the site of the injection: within the first hour after injection all of the material administered has entered into circulation. The decreasing blood level curve represents the falling concentrations of antibiotic previously absorbed, in process of elimination through the kidney. Quite different is the picture with repository treatment. The penicillin is continuously absorbed over long periods: the blood concentration at a given interval is a resultant of the end parts of blood level curves of previously absorbed penicillin and of the initial peak values of freshly absorbed penicillin curves. Thus, apparently identical blood level curves obtained with various vehicles might represent com-

pletely different absorption-elimination relationships. One might compare the repository injection to frequently repeated injections of decreasing dosages at very close—or zero—intervals. Some absorption of the repository penicillin continues, as was shown by us, even when the blood level is already negative, in contrast to aqueous penicillin where the absorption ends before the blood level does. This continuous absorption—which goes on even as late as 15 hours after negative blood level—is probably the main mechanism responsible for the increased retention in the organism and for the longer therapeutic utilization of repository penicillin.

Our experiments also indicate that penicillin injected in oil alone is absorbed with delay, even though the blood level is not significantly prolonged, compared to aqueous penicillin. In our opinion, it is this difference in absorption which accounts for the superior therapeutic results reported by various authors with penicillin in oil or in water-oil emulsions[3] which could not be due to the slight differences, if any[8,9], in the blood level duration.

Summary. It can be stated that the blood level curve alone is far from representing the various conditions related to therapeutic effectiveness, tissue retention, absorption, and so on. The prolongation of organ concentration, without noticeable increase of blood level duration, the more pronounced cerebrospinal levels, would suggest the advantages of higher dosage forms, irrespective of the bacteriological results on the "minimal effective" concentrations. This consideration should be applied to aqueous and repository treatment. On the other hand, it would appear that maintaining blood levels for equal treatment periods, repository penicillin (which in its absorption pattern very closely resembles continuously repeated aqueous injections) might result in a better therapeutic utilization than the aqueous injections given at intervals currently used.

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CHLORAMPHENICOL IN THE TREATMENT OF THE ACUTE MANIFESTATIONS OF BRUCELLOSIS*†

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During the period July, 1948, to April, 1949, an investigation of the antimicrobial therapy of typhoid, typhus and brucellosis was conducted in Guadalajara, Mexico. The results observed in a small group of patients with brucellosis who received aureomycin have been previously reported[1] and were in agreement with the observations of other investigators[5]. The present report consists of observations made in a series of 13 patients with the acute manifestations of brucellosis, all of whom received treatment with chloramphenicol.

Composition of Clinical Series and Methods of Study. Brucellosis is one of the common causes of febrile illness in Guadalajara and is associated with a high incidence of the disease among the cattle of the region[3]. The high rate of occurrence of the disease in humans is presumably related to the consumption of unpasteurized milk and contact with meat from these infected animals.

In Guadalajara, as in Mexico City[2], brucellosis is almost invariably the result of infection with *Br. melitensis.* All of 7 strains studied in this laboratory which were obtained from patients in Guadalajara have been identified as *Br. melitensis.* The disease is severe in this locality and is not infrequently associated with complications. The usual clinical manifestations observed are high fever, prostration, joint and muscle pains, night sweats, anemia and debilitation.

The present series consists of 11 patients who were treated in Guadalajara and 2 who were treated in the New York Hospital. Although the latter 2 patients were acutely ill, their infections were not as severe as observed in many of the Mexican patients. The group ranged in age from 17 to 67 years and consisted of 8 men and 5 women. Eleven of the 13 had been ill for 90 days or less at the time chloramphenicol therapy was started and the other 2 patients had been ill for periods of 7 and 10 months respectively. In 2 instances, acute febrile illnesses suggestive of brucellosis had occurred several months prior to the present episodes.

All of the patients were acutely ill and febrile prior to the start of therapy, although one patient (R. H.) was afebrile the day therapy was begun. The pertinent data con-

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cerning the patients is presented in Table 1.

Five of the present series were anemic with total red cell counts of less than 4 million. All of the patients exhibited a leukopenia or a normal total leukocyte count prior to therapy, and in most cases the differential count revealed a relative lymphocytosis. In 5 patients, the total erythrocyte counts were less

TABLE 1.—CHLORAMPHENICOL IN THE TREATMENT OF HUMAN BRUCELLOSIS

Pt.	*Sex*	*Age*	*Days ill before therapy*	*Chloramphenicol dosage*			*Days febrile after start of therapy*	*Herxheimer-like reaction*	*Course after treatment*
				mg./Kg./d.	*gm./d. (q 6 hr. doses)*	*No. days*			
W.L. (NYH)	M	28	13	25 12.5	2.0 1.0	10 14	5	No	Sustained remission, 4 mo.
F.S.	M	30	63	50	3.5	6	1	No	Sustained remission, 4 mo.
R.H.	M	17	26	50	3.0	6	Afebrile at start	No	Sustained remission, 4 mo.
Am.B.	F	29	10 mo.	100 25	5.25 2.6	5 5	2	Yes Mild	Sustained remission, 3 mo.
M.G.	F	67	85	100 25	4.0 1.0	2.5 7.5	1	Yes	Sustained remission, 4 mo.
L.R.	M	32	14	14 50	0.75 2.75	1 6	Cont'd fever	Yes	Incompl. remission with recurrent bacteremia, retreated then compl. remission 4 mo.
				100	5.5	6	4	No	
Al.B.	M	65	92	100 25	4.0 1.0	4 6	Cont'd fever	No	Incompl. remission, then recovery (not shown)
L.A.	F	60	7 mo.	40 100 25	2.0 5.0 1.5	1 3 4.5	2.5	No	Recurrent low fever and symp's, 13 days. No more treatment
P.R.	M	40	13	100 50	7.5 3.75	3.5 2.5	2	Yes	Recurrent low fever and symp's., 14 days. No more treatment
Ar.O.	F	29	62	40 200 100 25	2.0 10.0 5.0 1.0	1 1.5 3.5 4	1.5	No	Recurrent low fever and symp's., 60 days. No more treatment
A.C.	M	20	32	100 25	6.0 1.5	6.5 4	2	Yes	Severe relapse with orchitis, 10 days. Retreated with aureomycin. Remission sustained 2 mo.
Ca.A.	F	19	19	22 50	1.0 2.5	1 6	1	No	Febrile relapse, severe, 11 days. Retreated—sustained remission 3½ mo.
				100 25	5.0 1.25	4 6	2	No	
K.S. (NYH)	M	24	9	51	4.0	6	2	No	Prompt remission. Relapse after 26 days. Retreated with aureomycin

than 4 million per cmm. The total leukocyte and erythrocyte counts before and after therapy may be seen in Table 2.

The diagnosis was established by means of the demonstration of repeated high brucella agglutinin titers in all cases and the presence of bacteremia in 3 patients. The course of antibody titers and bacteremia throughout the illness are presented in Table 3. Four of the patients (F.S., R.H., A.C., J.R.) were ambulatory during therapy but their symptoms were of a severity which in most circumstances would have constituted an indication for hospitalization.

All were observed for 1 or 2 days prior to the start of therapy during which time several blood cultures were obtained. After chloramphenicol therapy was started, cultures were obtained daily during the febrile period in all patients. Duplicate specimens were routinely cultured in trypticase soy and liver infusion broth and were sub-cultured at frequent intervals for a period of 20 days. Blood cultures and serum specimens were obtained at intervals of 1 week and 1, 2 and 3 months after the completion of treatment and each day during the febrile period of a relapsing infection. The agglutinin titers were

TABLE 2.—CHLORAMPHENICOL IN BRUCELLOSIS

Cell Counts on Peripheral Blood*

	Erythrocytes Millions/Cu.mm.		*Leukocytes Millions/Cu.mm.*	
	Treatment		*Treatment*	
Patient	*Before*	*After*	*Before*	*After*
W.L. (NYH)	4.85	5.03	5.8	5.8
F.S.	5.8	—	6.6	—
R.H.	5.6	4.6	8.6	8.1
Am.B.	4.9	4.7	3.3	5.1
M.G.	3.95	—	6.0	—
L.R.	3.4	2.8	3.9	2.3
Al.B.	3.8	4.1	9.5	13.4
L.A.	4.6	—	4.1	—
P.R.	5.3	5.7	8.5	5.8
Ar.O.	3.2	2.9	7.5	6.7
A.C.	4.5	4.1	7.1	6.9
Ca.A.	3.2	3.9	6.2	8.7
K.S.	4.9	—	4.7	—

* Counts done within 2 days before and after treatment

TABLE 3.—ANTIBODY TITERS IN BRUCELLOSIS

		Weeks After Therapy						
Patient	*During Therapy*	*1–2*	*3–4*	*5–6*	*7–8*	*9–10*	*11–12*	*24*
W.L. (NYH)	1:10240		1:10240	1:5120			1:640	1:160
F.S.	1:2560	1:5120	1:2560		1:5120			
R.H.	1:640	1:640						
Am.B.	1:1280	1:1280	1:1280		1:1280		1:1280	
M.G.	1:320	1:320						
L.R.*	1:320	1:640	1:1280		1:640		1:2560	
Al.B.*	1:2560	1:2560	1:2560	1:5120		1:2560		
L.A.	1:640	1:640	1:320		1:160			
J.R.	1:640	1:5120	1:5120					
Ar.O.	1:5120	1:2560		1:2560		1:2560		
A.C.	1:1280	1:640	1:1280					
Ca.A.*	1:160	1:320		1:160		1:80	1:160	
K.S. (NYH)	1:640	1:1280	1:1280	1:1280				

* Bacteremic

initially obtained by the rapid slide agglutination technique described by Huddleson. The results presently reported, however, although in general in agreement with those observed by the slide method, were done in tube dilution and reported after overnight incubation. Chloramphenicol serum concentrations were determined by two-fold serial dilutions tests and were read after 24 hours incubation. The test organism used was the C203 strain of hemolytic streptococcus. These data are presented graphically in Fig. 1.

The identification of the strains of *Br. melitensis* isolated was based on the characteristic reactions in dye inhibition and hydrogen sulfide production tests, and agglutination in high dilution against brucella antiserum. The sensitivities of these strains to chloramphenicol, aureomycin and streptomycin on solid and liquid media after 72 hours' incubation were almost identical, being approximately 2 gammas per cc. for each agent. One strain (L.R.) obtained subsequent to a course of therapy was inhibited *in vitro* by the same concentration of chloramphenicol which had been inhibitory for the pretreatment strain isolated from the same patient.

Chloramphenicol*, produced by the fermentation process, was furnished in 0.25 gm. capsules for oral dosage. The individual regimens used may be seen in Table 1. In general, 50 to 100 mg. per kg. per day were administered during the febrile period and 25 mg. per kg. per day thereafter for a total period of 6 to 10 days, usually the latter. A few patients received smaller initial doses because of the possibility that an unduly severe exacerbation of the infection might occur at the start of therapy.

Results. The clinical course after the start of chloramphenicol therapy was regularly characterized by rapid improvement. About all of the patients were afebrile by the 2d or 3d day and all experienced great symptomatic improvement. Joint pains, asthenia, and other signs of chronic illness diminished in severity, but required a longer time to disappear completely. It was noteworthy that, by the finish of therapy, even the most acutely ill patients had gained enough strength to become ambulatory. In 2 of the 3 patients with bacteremia, however, the disappearance of all evidences of infection was

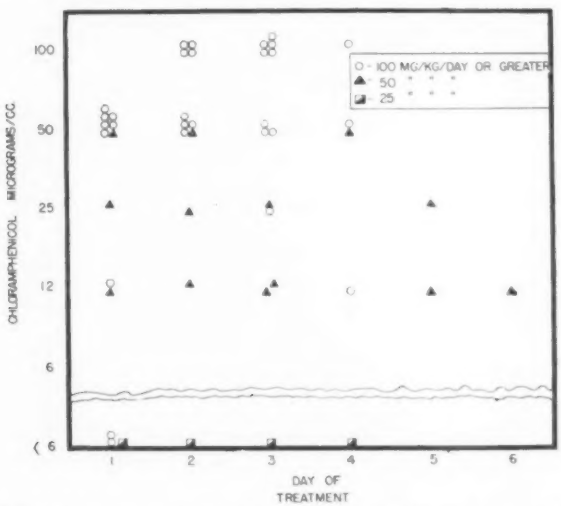

FIG. 1.—Chloramphenicol serum concentration in 12 (of 13) patients with brucellosis. High serum concentrations were maintained with doses of 50 mg. per kg. of body weight or more each day.

* Chloramphenicol (Chloromycetin®) was supplied through the courtesy of Doctor Elwood A. Sharpe, Parke, Davis and Company, Detroit, Michigan.

not complete at the end of the first course of chloramphenicol therapy. Moreover, the speed of recovery was slower in one patient who received only small daily doses of the drug (25 mg. per kg.). Even these differences were not marked, however, and the response of the others was remarkably uniform.

The results in the entire series are presented in 3 groups according to whether remission was sustained, incomplete, or followed by relapse.

Sustained Remissions. Five patients treated in New York Hospital and whose infection was presumably caused by a brucella strain other than *Br. melitensis*. He was the first patient in the series and received only 25 mg. per kg. of body weight per day of chloramphenicol because the possibility of an acute exacerbation of the infection was feared. On this low dosage, defervesence was slow and required 6 days for completion. It should be noted that this patient, unlike the remainder of the series, received therapy for a

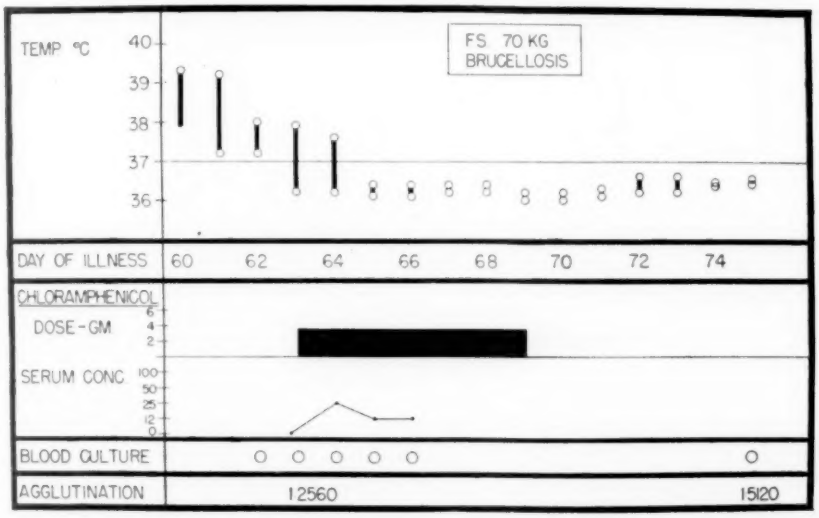

Fig. 2.—An immediate and sustained recovery following administration of chloramphenicol. This acutely ill patient was able to resume his work shortly after treatment.

(W.L., F.S., R.H., Am.B., M.G.) have had sustained clinical remissions since therapy with chloramphenicol. Brucella were not isolated from the blood of any of this group of patients, despite the fact that they were all febrile and acutely ill prior to therapy. There were no definite complications present among the group, and none was anemic, although all showed a relative leukopenia. The initial defervesence after the start of therapy was rapid in all but one of the patients. The exception was Patient W.L., who was total period of 24 days although during the last 18 days of treatment the total daily dose was only 1 gm. per day (12.5 mg. per kg.).

A representative example of the course after therapy observed in the remaining 4 patients whose remissions have been sustained may be seen in Fig. 2. This patient (F.S.) was a young man who had been well until 2 months before start of therapy. His temperature curve had been of the undulating type frequently observed in brucellosis and he had had especially severe joint

and muscle pains and sweating. He was not admitted to the hospital but had been forced to stop work because of his illness. All blood cultures were sterile, including 2 taken immediately before start of treatment. The abrupt defervescence in this patient was associated with similar clinical improvement. Shortly after stopping therapy he was able to resume his job and has remained well.

INCOMPLETE REMISSIONS. Two patients had incomplete remissions following therapy with chloramphenicol. Prior to the start of chloramphenicol therapy he had been acutely ill for 9 days, but the obvious appearance of severe chronic illness suggested that he had been ill for a longer period. His major complaints were weakness, generalized aches and pains, severe night sweats, chills, high fever and anorexia. Physical examination revealed a wasted, pale, prostrated male with a hacking cough and bilateral basal moist rales. Roentgenographic examination of the chest revealed no pulmonary or cardiac abnormalities and several specimens of sputum were negative on direct examination for acid fast organisms. As may be seen, *Br. melitensis* was repeatedly isolated from his blood. Anemia and leukopenia were present. He received 0.75 gm. of chloramphenicol

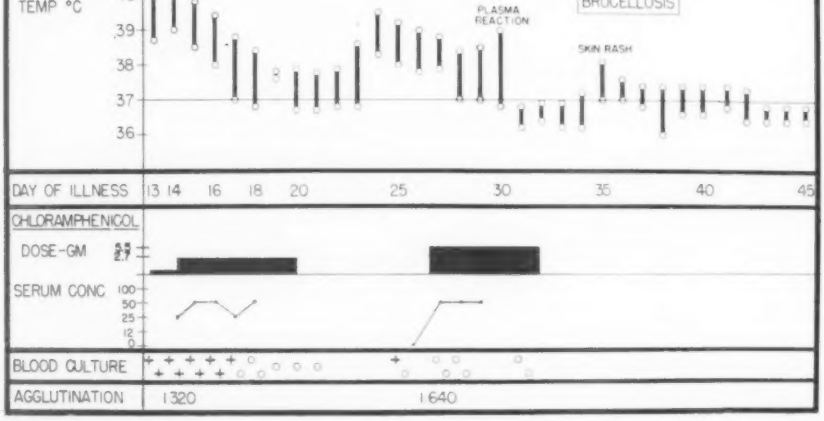

FIG. 3.—Course of brucellosis in a severely ill, bacteremic patient after chloramphenicol. Partial remission occurred but was promptly followed by clinical and bacteremic relapse. Retreatment with sustained recovery.

Bacteremia was present in both and they were the most severely ill patients in the entire series. A description of the course of the infection in both individuals is given because of the wide difference in the daily doses of drug administered.

Case Reports. CASE 1. L.R. received the smaller dose, and his course may be seen in Fig. 3. He was a 32 year old male who had had a previous acute episode of brucellosis 8 months before the present admission. At that time he had received high doses of streptomycin and sulfadiazine administered concurrently without appreciable effect. during the first 24 hours of therapy and 50 mg. per kg. per day thereafter for a total period of 6 days. It should be noted that bacteremia persisted for approximately 72 hours after the start of the antimicrobial therapy. Nevertheless, by the end of the 3d day of treatment the patient was improved with less pain and sweating, return of appetite and strength and his temperature was falling, although some fever persisted. Moreover, at the termination of therapy (after 6 days) he continued to have small elevations of temperature in the afternoon. During the first 48 hours after the cessation of therapy the patient's condition remained unchanged, but on the 3d day there was an appreciable increase in fever which was accompanied by

a return of bacteremia. Chloramphenicol was restarted in daily doses of 5.5 gm. (100 mg. per kg.) which was twice the dose the patient had previously received. The reinstitution of therapy with the increased dose was followed by rapid improvement, and, save for a transient but severe herpetic rash associated with low fever, the patient made a gradual and steady recovery. The complete remission of all signs of infection has been sustained.

CASE 2. The course of the other patient (Al. B.) in whom incomplete remission was also observed, may be seen in Fig. 4. At the start of therapy, this patient was extremely ill and bacteremia was present. He complained of severe backache and on physical examination an acutely tender area was present in the low back region. Roentgenographic examination revealed deformity, lateral calcification, and evidence of bony destruction in the first lumbar vertebra, presumably the result of the brucella infection. The chloramphenicol regimen used initially in this patient (100 mg. per kg.) was twice as large as the dose originally used in the previous patient in whom incomplete remission occurred. Moreover, in the present patient the antimicrobial therapy was continued for a total period of 10 days, during which time he improved rapidly. Some evidences of continued infection, principally low grade fever, were still present when chloramphenicol was discontinued, however, and within a few days his temperature had again risen to the pretreatment level. The administration of chloramphenicol, in the same doses, was immediately restarted and was followed by prompt recovery. The complete remission of this patient's illness has been maintained.

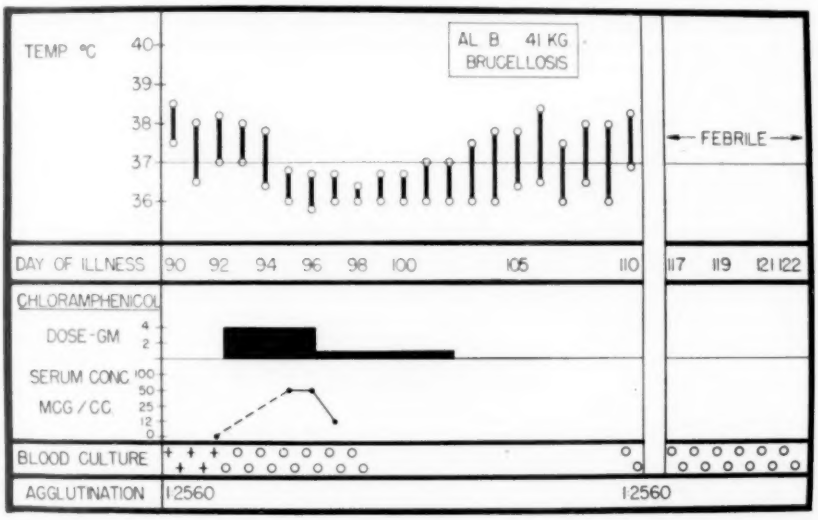

FIG. 4.—This patient, an old man, presented evidence of involvement of lumbar vertebrae in addition to his other clinical manifestations and bacteremia. Incomplete remission followed therapy with chloramphenicol. This patient was retreated later successfully (not shown).

RELAPSES. Relapse of the infection occurred in 6 of the 13 patients within 1 to 8 weeks after cessation of the antimicrobial therapy. The exact intervals between the completion of therapy and the appearance of relapse were: 8, 9, 13, 19, 26, and 60 days. The total period of chloramphenicol therapy in these 6 relapsing infections was 6 days in 2 patients and 7 to 10 days in the others (Table 1). Despite numerous blood cultures it was not possible to demonstrate bacteremia in any of these patients.

The relapses were mild in degree in 3 patients (L.A., J.R., A.O.) and were characterized by a return of joint symptoms, low grade fever, and night sweats. All 3 patients recovered with-

out receiving antimicrobial therapy. In the other 3 patients, however, the relapses resulted in a return of all the acute manifestations of the illness. In 2 instances, the relapsing infections were treated with aureomycin (because of a temporary shortage of chloramphenicol) but the 3d patient (Ca. A.) received a 10-day course of chloramphenicol (Table 1). In all 3 instances, including one patient with a severe orchitis (Fig. 5), there was a prompt defervescence and disappearance of symptoms and the complete recoveries have been maintained.

phenomenon. The most impressive instance of such a "Herxheimer-like" exacerbation may be seen in Fig. 5. The patient (A.C.) had been acutely febrile without bacteremia for 32 days before antimicrobial therapy was started. During the first 24 hours after the first administration of chloramphenicol (6.0 gm. daily) his temperature was a centigrade degree higher than previously and he complained of weakness, cardiac palpitation, and pain in the upper abdomen. These symptoms persisted for 48 hours at which time defervescence occurred. His sub-

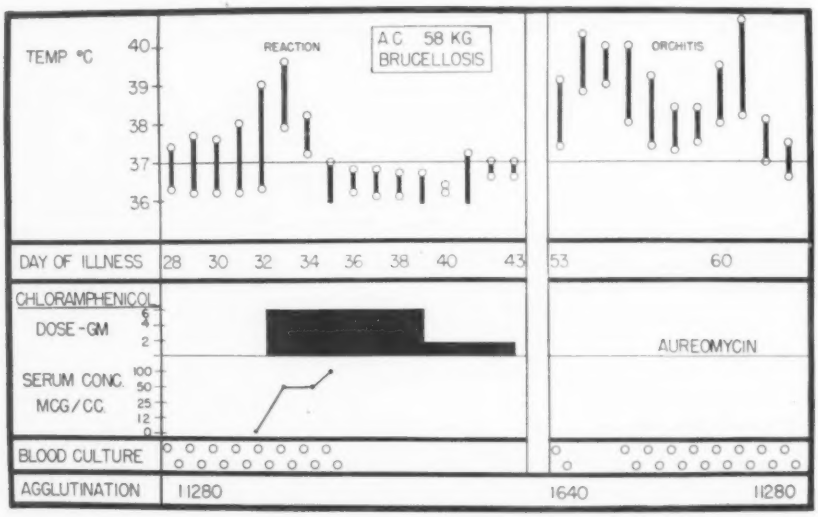

FIG. 5.—Abrupt rise in temperature with other evidences of a generalized reaction soon after initiation of chloramphenicol therapy. Clinical remission followed but relapse occurred in 10 days associated with a severe orchitis. Sustained recovery followed administration of aureomycin.

EXACERBATION OF ILLNESS AFTER START OF THERAPY. In 5 of the 13 patients, fever and associated symptoms were more intense during the first or second days of chloramphenicol therapy than during the pretreatment period (Table 1). In no instance was this exacerbation of the illness at all alarming and it was not possible to be certain that this intensification of the illness represented a drug-induced

sequent course during treatment was uneventful and he experienced the relapse of his infection accompanied by the severe orchitis which was mentioned previously.

An intensification of the illness after the start of therapy was noted in only 1 patient (L.R.) who received a preliminary low dose of the drug.

TOXICITY. Toxicity of serious consequence was not observed during or

subsequent to the administration of the chloramphenicol. Four patients complained of loose bowel movements, a reaction which was apparently attributable to chloramphenicol therapy. Nausea and vomiting occurred in 3 patients, and in 2 of these it was severe (Ar.O and J.R.). The former patient was receiving 200 mg. per kg. per day of chloramphicol, however, and her symptoms subsided when the dosage was reduced.

treatment was associated with return of blood counts to normal figures. It is not considered that these results indicated hematologic toxicity.

In general the brucella agglutinin titers remained elevated during a 6 to 12-week period of observation following the completion of treatment. A significant reduction (1:10, 240 to 1:160) of the agglutinin titer has occurred in one patient (W.L.) during the 6 months following the cessation of

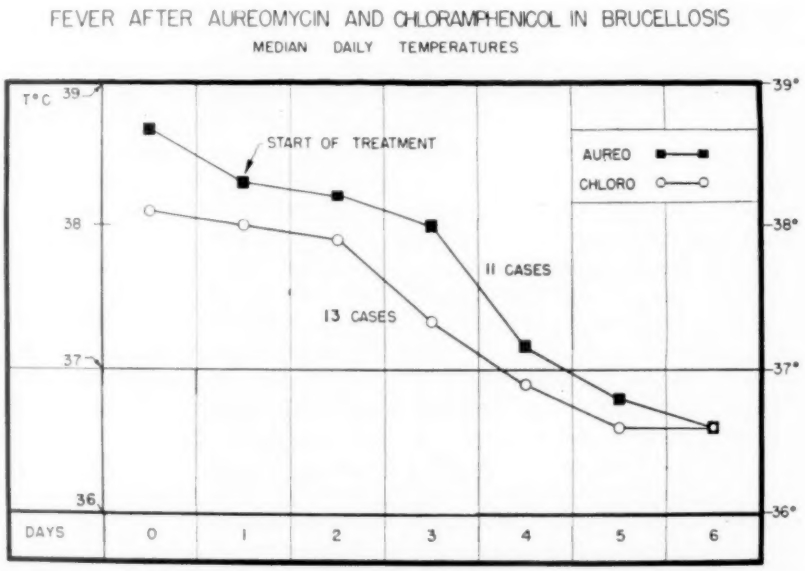

FIG. 6.—Febrile course after aureomycin and chloramphenicol in 2 groups of patients, most of whom represent infections with *Br. melitensis*. Prompt defervescence occurred in both groups and the differences that may be observed probably represent variations in case material.

As may be seen in Table 2, only slight changes were observed in the number of circulating erythrocytes and leukocytes immediately before and after therapy. In one patient (L.R.) a significant reduction in the total leukocyte count was observed immediately following the first course of therapy. This occurred, however, in the presence of a continued bacteremic brucella infection and subsequently rapid clinical improvement with retreatment. This patient contracted his disease in New York and presumably was an infection with *Br. Abortus* which is usually more susceptible to antimicrobial therapy than is *Br. melitensis*. Moreover, this patient is the only one in the series who received chloramphenicol for a total period of 24 days. There was no apparent correlation between the height of the antibody titer and the occurrence of relapse.

ABSORPTION OF CHLORAMPHENICOL. The concentration of chloramphenicol in serum specimens obtained at the time of daily blood cultures is shown in Fig. 1.

Different dosage regimens are indicated and the results are plotted according to the day of treatment. It may be seen that serum concentrations of 50 to 100 micrograms per cc. were regularly maintained on doses of 100 mg. per kg. per day or greater. Patients receiving 50 mg. per kg. per day maintained concentrations ranging from 12 to 50 micrograms p. cc. The one patient receiving only 25 micrograms p. cc. showed no measurable serum concentrations. These results are consistent with other studies on chloramphenicol absorption.

Discussion. The administration of a short course of chloramphenicol to 13 patients with the acute manifestations of brucellosis was invariably followed by rapid improvement, which in 11 patients continued to a complete remission of the illness. The improvement in the other 2 patients, though definite, was not as complete and a return of the acute illness occurred promptly when the antimicrobial therapy was discontinued. Moreover, in 6 other patients, the remission of the illness was not permanent and a relapse of the infection occurred 1 to 8 weeks following cessation of therapy. The 2 patients with incomplete remissions recovered promptly when retreated with chloramphenicol, as did the 1 patient with a relapsing infection who received a second course of the drug.

The *uniformity* with which the acute manifestations of the illness receded soon after the start of drug therapy was sufficiently striking to make it reasonable to believe that the effects observed were attributable to the drug.

Thus it appears that chloramphenicol exerts an impressive effect upon the course of acute brucellosis. The fact that a single 6 to 10-day course of treatment was followed by immediate recurrence in 2 instances and relapse in 6 others, presumably indicates that the particular regimen used was too short for ideal therapy. The prompt recovery which occurred in all patients who received additional treatment tends to support this assumption.

It is not surprising that such a short period of treatment as 6 to 10 days was followed by a high incidence of relapse. There is ample evidence that chloramphenicol, like other antimicrobial drugs, seldom actually eradicates an infection. Instead the administration of the drug serves to check the active progress of the infection until the forces of the host can be sufficiently mobilized to exert final control. If the host is unable to exert much control or if the artificial suppression of the infection is relieved prematurely, relapse of the infection will occur. In unpublished experiments in this laboratory[7] it was possible to culture *Br. melitensis* uniformly from the abdominal viscera of mice which had received 10-day courses of either chloramphenicol or aureomycin in doses of identical weight.

In an infection such as brucellosis, which is so frequently chronic, it would be most unusual if the host-parasite relationships were such that a short period of suppressive therapy would upset the balance in favor of the host with any degree of regularity. It is not yet established that the prolongation of antimicrobial therapy in brucellosis will appreciably reduce the incidence of relapse which has been noted following short courses of chloramphenicol or aureomycin. Nevertheless, as the emergence of drug-resistant microorganisms is apparently not to be feared, it would seem advisable to continue therapy for a total period of

4 to 6 weeks, in the hope that the incidence of relapse can be reduced to a minimum.

It is of interest to compare the results in the present series with the results observed after the concurrent administration of streptomycin and sulfadiazine, or after aureomycin therapy. Spink has observed in patients with *Br. abortus* infections, that 7 to 10 days were required for defervescence to occur after the start of combined streptomycin-sulfadiazine treatment[6]. When the same type of therapy was administered to patients with *Br. melitensis* infections, Spink and his associates[5] observed an even less satisfactory response. In contrast, he and his associates[5] and other investigators[4] have found that aureomycin is highly active against the acute form of the infection, whether caused by *Br. melitensis* or the other brucella strains.

The results observed from the use of aureomycin in brucellosis seem to be roughly comparable to the results in the present chloramphenicol series in that both drugs appear to be highly active in suppressing the acute manifestations of the infection. Consequently it is of interest to compare the median daily temperatures during treatment of an aureomycin treated group with the present chloramphenicol series. The aureomycin series consisted of 11 patients with the acute manifestations of brucellosis who were also treated in Guadalajara. Some of these cases have been included in a previous report[1]. All of these patients received aureomycin in doses which were the same or lower (by weight) than the chloramphenicol used in the present study. The median daily temperature of the two groups may be seen in Fig. 6. Despite the fact that the patients who received aureomycin exhibited a higher temperature before therapy, after 3 days of treatment no appreciable differences existed between the median temperatures in the 2 groups. It is considered that the slight differences which can be observed reflect differences in case material, but these were not statistically significant.

Similar data are not available from other series of patients treated with these agents. Woodward[8], however, has reported that the mean duration of fever of a group of patients with brucellosis (for the most part *Br. abortus* infections) treated with chloramphenicol was slightly more than 2 days.

From the report of Spink *et al.*[5] it was calculated that the mean duration of fever in patients with *Br. melitensis* infections treated with aureomycin was 3.5 days. It would appear that the longer period of fever in Spink's cases is a reflection of the severity of the infecting strain, rather than limitation of drug effect because of the persistence of fever in 2 patients in the present series of predominantly *Br. melitensis* infections past the period of drug therapy.

With due regard for the obvious uncontrolled variables in the above comparisons they fail to reveal a definite superiority of either drug in suppressing the acute manifestations of brucellosis. Moreover, it is doubtful whether a comparative evaluation of the 2 drugs on the basis of relapse ratio would have any significance, as the total number of treated cases is so small and regimens of various lengths were used.

There is some indication from the present study that daily doses of chloramphenicol which are less than 100 mg. per kg. (6 gm. in an adult of average size) are less than maximally effective during the acute phase of brucellosis. For example, recovery was delayed in one severely ill patient who received only 2.75 gm. daily (50 mg.

per kg.) and in a patient with a milder illness who received 2.0 gm. daily (25 mg. per kg.). It is not possible to state from this study whether daily doses larger than 100 mg. per kg. would serve a useful purpose. In the 1 patient who received 200 mg. per kg. (10 gm. daily) severe vomiting occurred.

The maximally effective daily dose of aureomycin in acute brucellosis has also not yet been established, although initially satisfactory results have been observed from the use of 2.0 gm. daily for a 9-to-15-day period[5]. It is to be hoped that with the increased availability of the 2 drugs it will be possible to conduct a comparative study of the question of the maximally effective daily dosage of each.

In the absence of such exact information, when chloramphenicol is to be used in the treatment of brucellosis, it would seem advisable to administer 100 mg. per kg. daily during the acute illness followed by 25 to 50 mg. per kg. thereafter for a total arbitrarily chosen period of 4 to 6 weeks. It is considered that patients whose weight is greater than 70 kg. need not receive more than 7 gm. daily during the acute illness and no more than 3 gm. daily during the remainder of the period of therapy. It is hoped that by such a prolongation of the total period of antimicrobial therapy, the incidence of relapse will be appreciably reduced.

Summary. The administration of chloramphenicol for a short period to 13 patients with the acute manifestations of brucellosis was followed in every instance by rapid clinical improvement. In more than one-half of the cases, however, permanent remissions of the illness failed to occur. It is believed that in an infection which may be as protracted as brucellosis, it is advisable to administer antimicrobial therapy for much longer than the 6-to 10-day period used in the present study.

Acknowledgement is gratefully made to Dr. Carlos Calderon of the University of Guadalajara for his assistance and the use of his laboratory in the conduct of this study.

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THE CARDIOVASCULAR EFFECTS OF THE INTRANASAL ADMINISTRATION OF ACETYL-BETA-METHYCHOLINE CHLORIDE (MECHOLYL)*

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Paroxysmal tachycardias of the supraventricular type are most effectively terminated by stimulation of the vagus innervation of the heart. This may be accomplished (1) mechanically, by carotid sinus or ocular pressure, and (2) chemically, by the administration of mecholyl subcutaneously or digitalis preparations intravenously. The disadvantage of these procedures is that they cannot be applied by the patient and require treatment by a physician or nurse. Since paroxysms of tachycardia occur at unpredictable intervals, it seemed advisable to ascertain whether the therapy could be modified so that it might be applied by the patient.

Mecholyl in large doses up to 1 gm. administered by mouth produces systemic reactions which are delayed and minimal in degree. The cardiovascular effects are so mild that a cardiac action of therapeutic value cannot be anticipated. In view of the rapid and active systemic reaction following the sublingual administration of nitroglycerine, it seemed possible that mecholyl, also a powerful vaso-dilating substance, might similarly be absorbed from the oral mucous membrane. Mecholyl is very hygroscopic and thus unsuitable for preparation in tablet form for sublingual administration. Acetyl-beta-methyl choline iodide was prepared in tablets of 25 mg. When this compound was administered sublingually in doses of 25 to 100 mg. no systemic reactions were noted. In view of the possibility that the inactivity of the sublingual method might result from the inactivation of mecholyl by the cholinesterase of the saliva, prostigmine bromide was added to the tablets. Following the sublingual administration of the mecholyl-prostigmine tablets, there was no evidence of any systemic reaction.

In 1937, Van Dellen, Bruger and Wright[6] observed that mecholyl was rapidly absorbed by the nasal mucosa when administered in a glycol or water

* This study was carried out with the support of the Dorothy H. and Lewis Rosenstiel Foundation.

vehicle. Doses of 25 to 100 mg. were given through a glass dropper in 11 patients with a tendency to hypertension. They noted that general reactions such as sweating, salivation, lacrimation and drop in blood pressure were frequently quite marked. These observers, however, did not make any direct comparisons of the reactions following the intranasal and subcutaneous routes of administration. The purpose of the present study was to determine whether the cardiovascular effects of a therapeutic subcutaneous dose of mecholyl could be produced by intranasal administration.

Early Procedure. A comparison was made of the depressor reactions to the subcutaneous and nasal administrations of mecholyl in a group of hypertensive patients. The observations with each method of administration were usually carried out on successive days. The subcutaneous dose was 25 mg. For the intranasal route, a pledget of cotton of suitable size for intranasal application was placed on a wooden applicator. Mecholyl, 200 mg., was dissolved in a small amount of water, just sufficient to saturate the cotton.* The

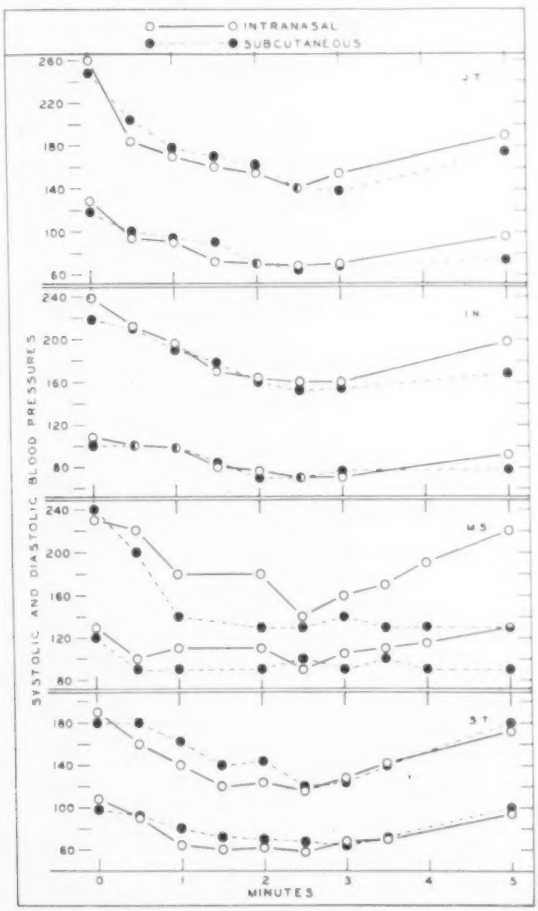

FIG. 1.—The effect on systolic and diastolic pressure of the intranasal and subcutaneous administrations of mecholyl in 4 hypertensive patients.

* The solutions were made of mecholyl crystals marketed in 1 and 10 gm. bottles for the preparation of solutions for iontophoresis. The mecholyl was supplied by Merck and Company.

applicator was then inserted well up into one nostril. The applicator was removed after approximately 2 minutes, when the flushing and sweating of the face became quite intense. It should be pointed out here that the amount absorbed at this time could not be estimated but that it was probably much less than the 200 mg. applied. This study does not attempt to compare the efficiency of subcutaneous and nasal administration. The purpose is merely to determine whether the effects of a therapeutic subcutaneous dose could be reproduced by intranasal application. Several blood pressure readings were made prior to the administration of the drug and readings were made at 30-second and 1-minute intervals, following the therapy by the subcutaneous and nasal routes. Pulse rates were also observed and in many instances electrocardiographic records were made. The onset and intensity of the systemic reactions such as flushing of the face, salivation and lacrimation were noted. Although these effects were quite marked and sensations of weakness, nausea and palpitation were occasionally observed, no alarming reactions were noted. It was not necessary to administer atropine to terminate the effects in any case.

Early Results. Mecholyl was administered by the 2 routes in 14 hypertensive patients. Fig. 1 shows typical responses in 4 patients. The depressor responses were as great and in some instances more marked following the intranasal application. A marked drop in blood pressure was usually noted at 30 seconds by both methods and at this interval, the intensity of the reaction

TABLE 1.—THE MAXIMUM FALL IN SYSTOLIC AND DIASTOLIC PRESSURE AFTER THE INTRANASAL AND SUBCUTANEOUS ADMINISTRATIONS OF MECHOLYL IN 14 HYPERTENSIVE PATIENTS

The Depressor Effects of Mecholyl Administered Intranasally and Subcutaneously.

Intranasal			*Subcutaneous*		
	Maximal Fall m.B.P.			*Maximal Fall m.B.P.*	
Initial B.P.	*Syst.*	*Diast.*	*Initial B.P.*	*Syst.*	*Diast.*
260/128	120	60	248/118	110	50
238/108	78	38	218/100	66	30
210/120	70	42	210/112	70	42
220/108	80	28	208/100	68	14
160/100	72	40	170/115	70	45
220/108	80	28	212/104	72	24
210/130	56	46	196/110	38	26
200/104	70	34	196/102	56	32
200/104	110	44	218/110	100	50
190/108	74	50	180/98	60	34
230/130	90	40	240/120	110	50
214/120	84	40	200/128	80	43
230/140	70	50	240/140	110	50
250/160	70	40	270/180	115	80

TABLE 2.—THE MAXIMUM FALL IN SYSTOLIC AND DIASTOLIC PRESSURES AFTER THE APPLICATION BY DROPPER INTRANASALLY OF 50 MG. OF MECHOLYL AND THE SUBCUTANEOUS ADMINISTRATION OF 20 MG. OF MECHOLYL

Intranasal			*Subcutaneous*		
	Maximal Fall m.B.P.			*Maximal Fall m.B.P.*	
Initial B.P.	*Syst.*	*Diast.*	*Initial B.P.*	*Syst.*	*Diast.*
200/100	50	30	195/110	65	40
195/85	50	5	196/120	45	10
148/60	38	5	140/60	35	10
150/80	45	20	150/70	55	25
190/120	25	30	190/120	65	50
145/70	35	10	145/70	35	15

was greater in 11 of the 14 cases by the intranasal route. The duration of the effect was usually longer by the subcutaneous method. Table 1 shows a comparison of the maximum depressor responses by the 2 routes of administration.

In these experiments no actual comparison could be made of the effects of a dose given subcutaneously with that

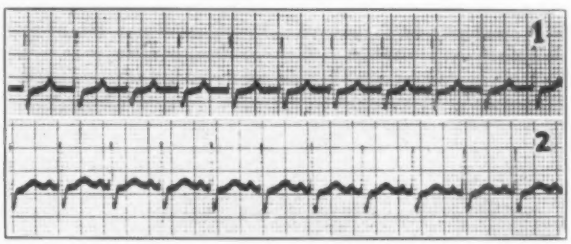

FIG. 2.—Upper strip shows a nodal tachycardia, rate 130. Lower strip taken 2 minutes after the intranasal application of mecholyl shows a sinus rhythm.

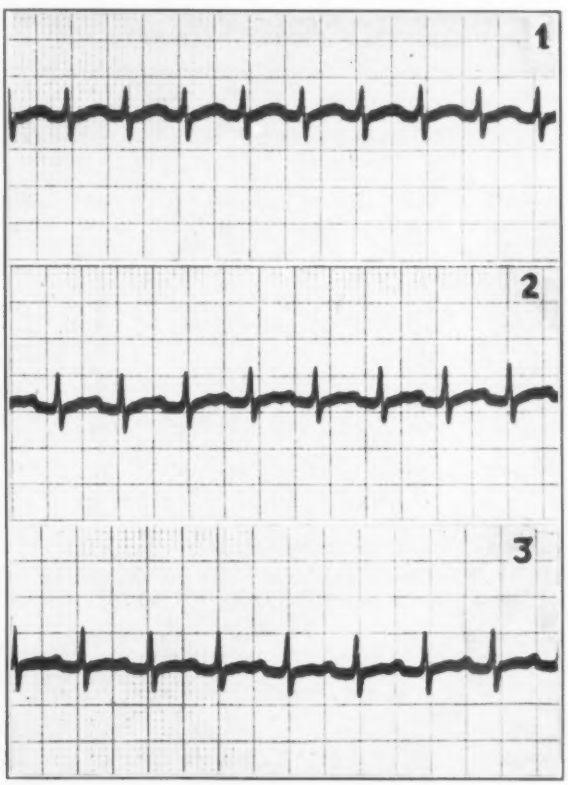

FIG. 3.—Upper strip shows supraventricular tachycardia rate 180. Middle strip taken 60 seconds after the intranasal application of mecholyl shows the beginning of the appearance of the P wave. Lower strip shows a sinus rhythm 90 seconds after the application of mecholyl.

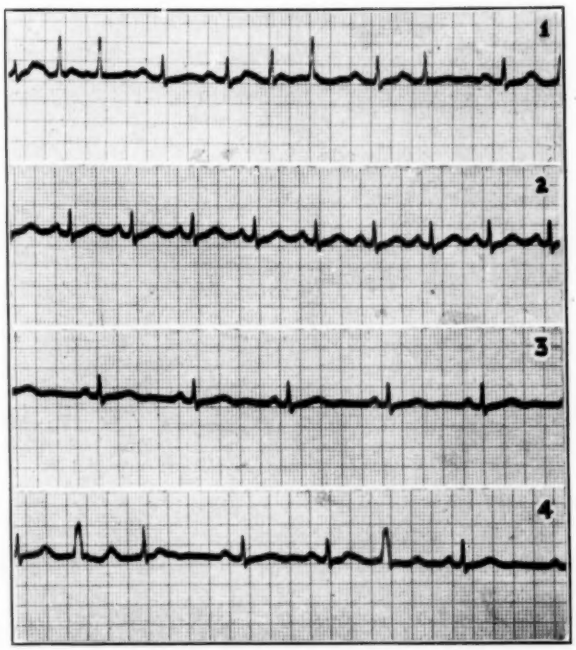

FIG. 4.—Upper strip 1 shows rhythm consisting predominantly of multifocal supraventricular ectopic beats. Strips 2 and 3 taken 2 and 3 minutes after the intranasal application of 200 mg. of mecholyl shows a sinus rhythm. Strip 4 taken 10 minutes after the administration of mecholyl shows the return of ectopic supraventricular beats.

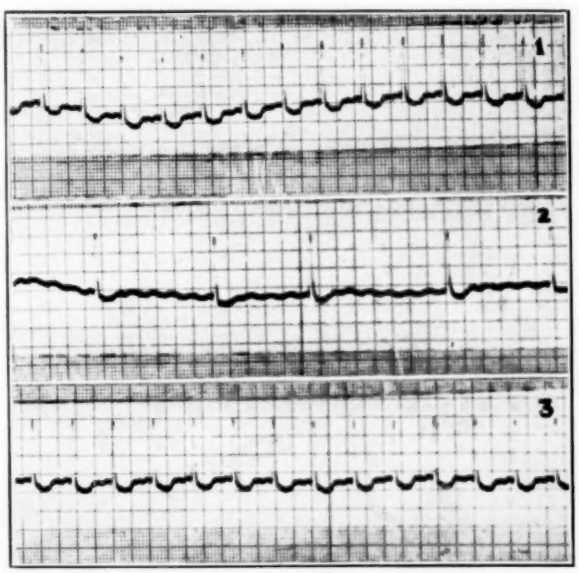

FIG. 5.—Upper strip shows an ectopic tachycardia, rate 136. This record could be interpreted as a supraventricular tachycardia or as a 2:1 auricular flutter. Pressure on the carotid sinuses had no effect. The diagnosis is established in the middle strip taken 90 seconds after the intranasal application of mecholyl, showing auricular flutter with marked slowing of the ventricular rate and irregular conduction. Lower strip taken 5 minutes after the administration of mecholyl shows the return of the rapid ventricular rate.

administered intranasally, since the amount of the drug absorbed at the time the nasal applicator was removed could not be estimated. In the following experiments, the drug was administered intranasally with a glass dropper, using a technique by which most or all of the drug was absorbed from the nasal mucous membrane.

Later Procedure. Six hypertensive patients were studied. After 3 control readings of the pressure were made, 20 mg. of mecholyl was administered subcutaneously and blood pressure readings were made at 1-minute intervals for 10 minutes. At a later period, these observations were repeated after the instillation into the nostrils of 50 mg. of mecholyl with a glass dropper. The solution for intranasal application was prepared so as to contain 25 mg. per drop and 1 drop was instilled into each nostril. Each patient was instructed to lie on his back with the head hyperextended. The patient remained in this position for several minutes after the instillation of the drug so as to favor complete absorption of the drug from the nasal mucous membrane.

The usual systemic reactions of warmth of the face, sweating, salivation and lacrimation occurred within 1 minute after both methods of administration. The onset, intensity and duration of the depressor reaction were

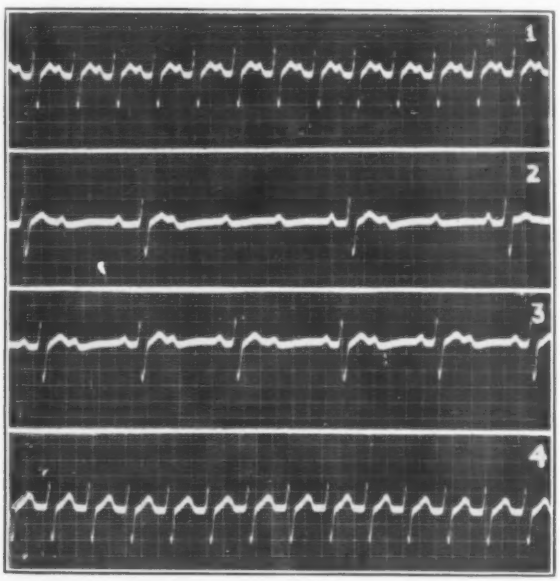

FIG. 6.—Strip 1 shows a sinus tachycardia. Strips 2, 3 and 4 show varying degrees of auriculoventricular block following the intranasal application of 200 mg. of mecholyl. Strip 4 shows prolongation of the P-R interval.

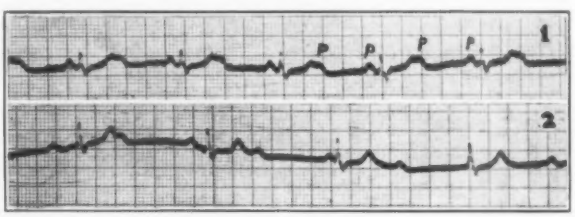

FIG. 7.—Upper strip shows 2:1 auriculo-ventricular block. Lower strip taken 2 minutes after the intranasal application of 200 mg. of mecholyl shows complete auriculo-ventricular block.

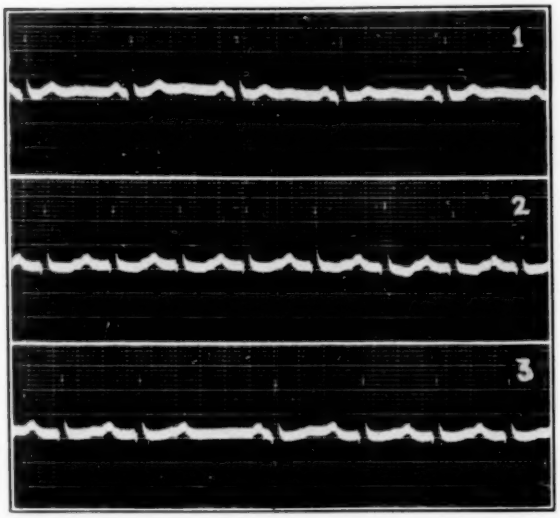

FIG. 8.—Strip 1 shows a heart rate of 68 and P-R interval of 0.16 second. Strip 2, taken 1½ minutes after the intranasal application of 200 mg. of mecholyl, shows a heart rate of 108 and P-R interval of 0.28 second. Strip 3 taken 2 minutes after administration of mecholyl shows prolonged P-R and occasional dropped beats resulting in 2:1 block.

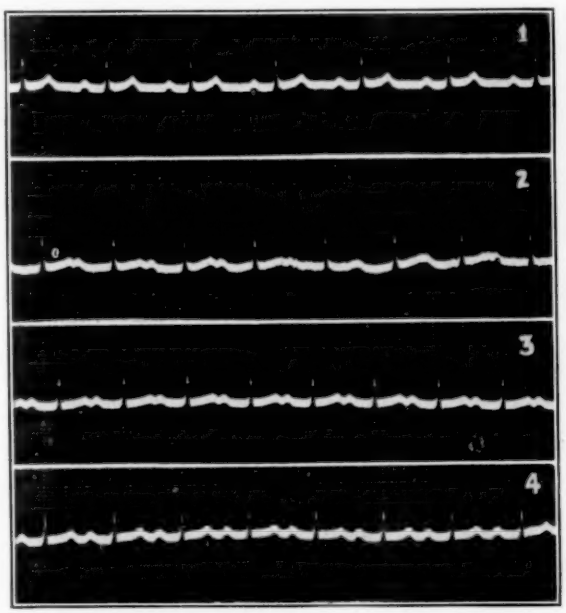

FIG. 9.—Upper strip shows heart rate 74 and P-R interval 0.2 second. Strip 2, taken 1½ minutes after the intranasal application of 200 mg. of mecholyl shows a rate of 94 and P-R interval of 0.36 second. Strip 3, taken 2 minutes after the application of mecholyl, shows a rate of 100 and P-R interval of 0.32 second. Strip 4, taken 2½ minutes after mecholyl, shows a rate of 90 and P-R interval of 0.28 second.

very similar in 5 of the 6 patients by both routes (Table 2). In 1 patient there was a greater drop in blood pressure following the subcutaneous administration. These observations suggest that 2½ to 3 times the amount of mecholyl is required by the intranasal route to reproduce the effects of a subcutaneous dose.

CARDIAC EFFECTS. Mecholyl by subcutaneous injection usually increases the rate of the heart[5] and this effect was observed in most instances in the present study. Electrocardiograms of patients with sinus rhythm usually show only a sinus tachycardia and occasionally depression of the T waves[4]. Occasionally a prolongation of the P-R interval is seen and rarely a transient complete block. In our electrocardiographic studies after the intranasal administration of mecholyl, the following cardiac effects were noted: termination of a nodal tachycardia (Figs. 2 and 3), the elimination of multiple auricular ectopic beats (Fig. 4), the induction of a high degree of auriculo-ventricular block in auricular flutter (Fig. 5), 2 instances of partial and complete auriculo-ventricular block (Figs. 6 and 7), 3 of marked prolongation of the P-R interval (Figs. 8 and 9), and 2 of transient auricular fibrillation (Fig. 10).

The induction of transient auricular fibrillation by the intranasal administration of mecholyl is of interest. Cohn and Macleod[1] reported auricular fibrillation in pithed bullfrogs following the intravenous administration of mecholyl. Iglauer, Davis and Altschule[2] produced auricular fibrillation and heart block in intact and unanesthetized dogs and rabbits by the intravenous injection of mecholyl. Nabum and Hoff[3] administered mecholyl subcutaneously to 5 patients, 4 having definite hyperthyroid-

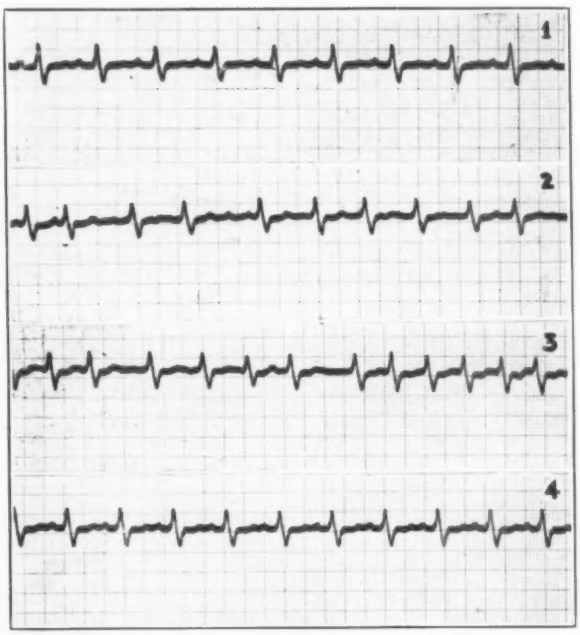

FIG. 10.—Strip 1 shows regular rhythm with a sinus tachycardia. Strips 2 and 3 show auricular fibrillation 2 minutes after the intranasal application of 200 mg. of mecholyl. Strip 4 shows sinus rhythm, 1 minute after the intravenous injection of 0.001 gm. atropine sulphate.

ism, the 5th patient being a doubtful hyperthyroid. In the 4 definitely hyperthyroid patients, the drug produced a short period of auricular fibrillation, but this was not observed in the doubtful case. Fairly large doses of the drug were used. These observers as well as others conclude that vagus activity is an important factor in the genesis of auricular fibrillation.

Discussion. The present study indicates that mecholyl is promptly and effectively absorbed from the nasal mucous membrane. It is also demonstrated that the cardiovascular effects of a therapeutic subcutaneous dose may be reproduced by the application of a larger dose intranasally. Although these results suggest a procedure for self medication of paroxysmal tachycardia, it is evident that such therapy is open to considerable objection. Mecholyl is a potent drug occasionally resulting in alarming reactions which may require antidotal therapy with atropine. In our experience, the reactions to the drug following the intranasal application have been moderately intense, but none of these could be classified as severe or alarming. This is probably due to the fact that the intensity of the reaction could be regulated by the removal of the applicator. It is possible that under certain conditions, self medication might be feasible: 1, in the situation where attacks are frequent, prolonged, disabling and resistant to more simple forms of therapy; 2, when the patient is sufficiently intelligent to follow the simple instructions, especially as to the removal of the applicator at the proper time; and 3, when the patient shows no unduly intense reaction after a trial administration of the drug by a physician. It should be pointed out that the intranasal route may be of some advantage in the administration of the drug by the physician. This method eliminates the necessity for the preparation of a sterile solution and for the use of a hypodermic injection. In our studies, the required amount of mecholyl was placed in small glass tubes. At the time of administration, a small amount of tap water sufficient to saturate a cotton pledget was added and the cotton on the applicator saturated with the solution and applied. In addition to the simplicity of administration, an advantage is that the intensity of the reaction can be regulated by the time that the applicator is permitted to remain in place.

This procedure also provides a simple method for the observation of the effect of a sudden drop in blood pressure on certain symptoms in hypertensive patients. In a small group of hypertensive patients, the influence of intranasal mecholyl was observed on the symptoms of headache, dizziness and head noises. In most instances, these symptoms were not influenced by the depressor reaction.

Conclusions. The depressor responses in hypertensive patients after the application of mecholyl intranasally are as prompt and as intense as those following the subcutaneous injection of a therapeutic dose of the drug.

Vagal effects on the heart can be demonstrated after intranasal application.

These results suggest that mecholyl by the intranasal route may be applicable in cardiac therapy.

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GASTRIC MUCOSAL BIOPSY FINDINGS CORRELATED WITH GASTROSCOPIC DIAGNOSES: PRELIMINARY REPORT BASED ON 50 PATIENTS

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The introduction by Wood and coworkers[2] of a gastric mucosa biopsy tube whose grasping mechanism is based on the principle of suction has made it possible to obtain excellent full-thickness mucosal specimens with a certainty, safety, and ease not hitherto possible. The instrument appears to offer important advantages as a clinical research tool[3], although, because the biopsy must be taken rather blindly and because only a relatively small portion of the gastric mucosa is made available for study, the method is expected to be of limited use in practical clinical diagnosis. An immediately evident useful function is that of further elucidation of the histopathology of the gastritides, and, for the gastroscopist, the confirmation or repudiation of the validity of his diagnostic ability in these diseases.

The Instrument. Using Wood's[2,3] description, a satisfactory biopsy tube was fashioned easily from odds and ends with simple handtools. A few variations in design were thought advisable. The distal inch was cut from the end of an Ewald tube, and a metal tube (outside diameter 7.5 mm., inside diameter 4.5 mm.) 42 mm. long was inserted a few mm., being held firmly in this position by the grasp of the Ewald tube. A rubber finger from a gastroscope bulb was forced a few millimeters into the other end of the metal tube and secured. An oval opening 3 x 3.5 mm. was filed through the side of the metal tube at about its mid-point, thus producing a cutting edge flush with its bore. A metal rod 10 mm. long, of such a diameter that it fitted snugly but slid easily within the metal tube, was excavated at one end with a conical dentist's drill so that a terminal cylindrical cutting edge was produced. The rod was attached by a small screw at the center of its excavated end to a long wire which ran back through the Ewald tube, to emerge through the wall of the latter near its end. A 30 cc. syringe attached to the proximal end of the Ewald tube completed the instrument.

Technique. In this clinic a biopsy has recently been obtained immediately prior to each gastroscopy; the contraindications to both procedures are the same. Following throat anesthetization, the biopsy tube is passed with the patient in the sitting position, and the stomach is drained through it as is routinely done before gastroscopy. By judging the depth of introduction and governing the direction of the tube's cutting hole, it has been found possible to select within a few centimeters, as checked later with the gastroscope, the region biopsied. An assistant makes sudden suction with the syringe and immediately indicates whether resistance is felt. If it is, the wire controlling the cutting cylinder is quickly pulled up a centimeter, cutting off the knuckle of mucosa which has been drawn through the hole in the side of the metal tube. The instrument is withdrawn and the specimen placed in fixative. It has been found that, unless the specimen is cut off as soon as suction is applied, histologic examination will show congestion of the mucosal vessels and hemorrhagic extravasation beneath the surface epithelium. About 5 seconds elapse between cutting of the biopsy and its fixation. Ninety-three satisfactory full-thickness mucosal specimens were obtained during the first 100 attempts at biopsy.

After the biopsy is taken, the patient is placed in the left-lateral position, and gastroscopy carried out. The exact location of the biopsied area is noted, as well as the apparent health of the mucosa in the region, for later comparative purposes. The biopsied area has usually been found without difficulty, appearing as a dark purple oval spot. No bleeding has ever been seen from the area itself, although mucus in the region is often blood-stained.

The Specimens. These are remarkably uniform in size and shape, appearing grossly as oval discs, 4 x 3 x 1.5 mm. Histologically it is found that, in addition to full mucosal thickness, a small portion of the submucosa is obtained. The greatest advantage of the method appears to be the excellence of cytologic preservation throughout the section; details in this regard will not be reported at this time.

Safety of the Procedure. Because of the construction of the instrument, with the cutting edges operating within the tube rather than externally as unguarded snippers, perforation of the gastric wall is hardly possible. The anatomic peculiarity of mobility of the gastric mucosa on the submucosa, furthermore, assures that tissues no deeper than the superficial submucosa be sucked into the instrument and severed from the stomach wall. No evidence of untoward result was found among the 100 cases thus far biopsied. Stools were examined for occult blood for 4 successive days in each of 23 patients whose stools had been guaiac-negative prior to biopsy, and none became positive. A two-thirds gastrectomy for benign antral ulcer was done on 1 patient 9 days following biopsy of the distal posterior wall of the pars media; a careful scrutiny of the resected specimen failed to reveal any defect to indicate the biopsy site.

Results. There were certain difficulties in categorizing the histologic pictures into a scheme comparable to the gastroscopist's classification, in spite of the assistance afforded by Schindler's[1] excellent monograph—a matter which, it is hoped, the biopsy tube may be effective in further elucidating at some future time. The assistance of Major Helmuth Sprinz, Chief of Laboratory Service, provided an authoritative interpretation of the histopathologic findings.

TABLE 1.—CORRELATION OF GASTROSCOPIC AND BIOPSY FINDINGS: 50 PATIENTS.

	Histologic picture				
Gastroscopic diagnosis	*normal mucosa*	*acute focal gastritis*	*chronic superficial gastritis*	*chronic atrophic gastritis*	*chronic hypertrophic gastritis*
Normal mucosa	23	1		1	1
Acute exogenous gastritis		5			
Chronic superficial gastritis	1		5		
Chronic atrophic gastritis				4	
Chronic hypertrophic gastritis	1				8

Correlation of histologic diagnosis and the gastroscopic findings at the area of biopsy (Table 1) revealed, in 50 patients, a 10% error in agreement. It is seen from the table that, although in no case was one type of gastritis confused gastroscopically with another, errors of both omission and commission were made in the endoscopic diagnosis of the normal mucosa.

Conclusions. 1. Experience with 100 patients indicates that the gastric mucosa biopsy tube described by Wood and collaborators is a safe and efficient instrument, that its clinical use is easy and practical, and that the biopsy specimens provided are satis-

factory for detailed investigative purposes.

2. Comparison of the histologic pictures with the gastroscopic diagnoses (at the biopsy sites) revealed a 10% gastroscopic error in 50 consecutive dyspeptic patients.

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THE EPINEPHRINE TEST FOR CORTICO-ADRENAL RESERVE FUNCTION AND THE EXCRETIONS OF CORTICOSTEROIDS AND 17-KETOSTEROIDS IN CHRONIC ULCERATIVE COLITIS

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Chronic ulcerative colitis may be markedly debilitating. In its advanced stages and its severe form it may result in overwhelming stress to the person afflicted. Such stress may be expected to affect the function of the adrenal cortex.

Hills, Forsham and Finch[3] found that after the injection of adrenocorticotrophic hormone in persons without evidence of impaired adrenal function, changes occurred in the levels of circulating leukocytes. The most definite response was found to be reflected by the eosinophils, their values diminishing on an average 73% from the initial level, with a range of 49% to 99%. This effect was considered to result from the release of cortico-adrenal hormones with an oxygen atom at position C-11. Patients with Addison's disease exhibited an average change in circulating eosinophils of only —4% from control values, with a range of +20% to —38%. This phenomenon has been elaborated into a test for adrenal cortical insufficiency by Thorn and his associates[5]. The level of the circulating eosinophils appears to be unimportant in governing the response[3,5].

Laragh and Almy[4] made the interesting observation that results similar to those described in the preceding paragraph may be obtained by the injection of epinephrine or insulin, as well as by surgical procedures. The response is presumably the result of pituitary stimulation of the intact adrenal cortex, with the release of corticosteroids. All control subjects in this series exhibited a drop in circulating eosinophils of more than 50% from control levels. No significant response was obtained in patients with adrenal or pituitary insufficiency. Of 3 patients suffering from severe chronic ulcerative colitis, 2 showed an actual increase in eosinophils after the injection of insulin, while the third exhibited a drop of only 12%. Normal responses were obtained in 5 patients with mild forms of the disease. It was suggested by Laragh and Almy that adrenal insufficiency may occur in patients with chronic ulcerative colitis.

We have employed the epinephrine

test as described by Laragh and Almy as a method of assessing corticoadrenal reserve function in patients with various degrees of chronic ulcerative colitis. The erythrosedimentation rate and the urinary excretions of corticosteroids and 17-ketosteroids were also determined in the majority of patients. The results were compared with those of the epinephrine test. It is our purpose to present the results of these tests.

Method. EPINEPHRINE TEST. All food and medication was withheld for at least 8 hours preceding the test and until the procedure was completed. Water was allowed ad libitum. At 8:00 a.m. blood was obtained by venipuncture and oxalated. Immediately, 0.5 cc. of a 1 to 1000 solution of epinephrine was injected subcutaneously. At 12:00 noon a second sample of blood was obtained by venipuncture and oxalated. Absolute eosinophil counts were performed on each of the two blood samples. The values obtained were compared and the difference was expressed as the percentage change from the control level.

The sedimentation rate (Westergren) was determined in all patients. The urinary 17-ketosteroids were assayed by the method of Callow, Callow and Emmens[1]. The Corcoran and Page[2] method was employed to ascertain the values of the urinary corticosteroids. The 17-ketosteroids and corticosteroids were determined on specimens of urine collected during the 24 hours preceding the epinephrine test.

Material. Ten young male physicians in good general health served as controls for the epinephrine test.

There were 14 patients suffering from chronic ulcerative colitis. These persons were classified as to degrees of clinical severity on the following basis: Group A: patients well enough to be ambulatory—2; Group B: patients requiring hospitalization, but with relatively mild symptoms—3; Group C: hospitalized patients with moderately severe symptoms and toxicity—3; Group D: hospitalized patients with severe symptoms and toxicity—6.

OBSERVATIONS: The results are summarized in Tables 1 to 6.

Epinephrine Test. As shown in Table 1, the eosinophil counts of 9 of the 10 control subjects decreased 50% or more from the initial level. The decrease ranged from 50% to 82%, with an average of 64%. The tenth subject was tested while suffering from a moderately severe, though not incapacitating, upper respiratory infection. In this subject the decrease in the circulating eosinophils was 38%. Two weeks later he still showed objective and subjective signs of illness; the eosinophil response was found to be —30%. Because of the presence of the upper respiratory infection this individual probably should not be considered as a "normal" control subject. It will be seen that one week after recovery from this infection, the eosinophil response was —49%.

TABLE 1.—EPINEPHRINE TEST: CONTROL SUBJECTS (ALL MEN)

Number	*Eosinophil change, per cent*
1	–53
2	–72
3	–71
4	–82
5	–59
6	–52
7	–50
8	–80
9	–61
10*	–38
10†	–30
10‡	–49

* Studied during upper respiratory infection

† Two weeks after first study, still with active infection

‡ One week after recovery

On the basis of these 10 control cases, we have divided the response to the epinephrine test into 3 groups: (1) responses of —50% or more: normal; (2) responses of —49% to —30%: questionably abnormal; (3) responses less than —30%: abnormal.

The results obtained from the 14 patients studied are summarized in Table 2. A definitely abnormal response was elicited in 5 patients, ques-

tionably abnormal results were noted in 5, and normal findings were present in 4 patients. Four of the 6 patients who were most seriously ill (Group D) showed definitely abnormal responses and the other 2 patients of this group gave questionably abnormal responses. On the other hand 1 ambulatory patient (Group A) was found to have an abnormal response to the epinephrine test. Of the 5 patients in whom a questionably abnormal response was obtained, 2 were from Group D, 2 were from Group C and 1 was from Group B. Four patients exhibited a normal response to epinephrine, 1 from Group C, 2 from Group B and 1 from Group A. There appears to be a rough parallelism between the degree of clinical severity and the response to the epinephrine test.

TABLE 2.—EPINEPHRINE TEST: COMPARISON WITH DEGREE OF CLINICAL SEVERITY

Number	*Sex*	*Epinephrine test, eosinophil change, per cent*	*Clinical group**
		DEFINITELY ABNORMAL	
1	M	+20	D
2	F	+ 0.4	D
3	F	− 0.3	D
4	M	− 0.6	A
5	M	−23	D
		QUESTIONABLY ABNORMAL	
6	M	−30	D
7	M	−43	C
8	M	−43	C
9	M	−44	B
10	F	−46	D
		NORMAL	
11	M	−54	C
12	F	−65	A
13	M	−66	B
14	F	−72	B

* A, ambulatory; B, hospitalized with minimal symptoms; C, hospitalized with moderate symptoms and toxicity; D, hospitalized with severe symptoms and toxicity.

Urinary Corticosteroids. Normal values in our laboratories for urinary corticosteroids range from 0.3 to 0.8 mg. per 24 hours, with no apparent differences between the sexes. Although an extensive series of determinations has not been made on the urine from normal subjects it is probable that the upper limit of normal for persons at rest in bed is not more than 1 mg. per 24 hours. The urinary excretion of corticosteroids was determined in 11 of the 14 patients. The values obtained are shown in Table 3, and are compared with the results of the epinephrine test.

TABLE 3.—URINARY CORTICOSTEROIDS: COMPARISON WITH EPINEPHRINE TEST AND CLINICAL GROUP

Number	*Sex*	*Urinary corticosteroids, mg. per 24 hr.*	*Epinephrine test, eosinophil change, per cent*	*Clinical group*
		BELOW NORMAL		
10	F	0.18	−46	D
2	F	0.26	+ 0.4	D
		NORMAL		
11	M	0.32	−54	C
14	F	0.39	−72	B
9	M	0.85	−44	B
6	M	0.88	−30	D
8	M	0.88	−43	C
4	M	0.96	− 0.6	A
		PROBABLY ELEVATED		
1	M	1.04	+20	D
3	F	1.12	− 0.3	D
5	M	1.48	−23	D

Five of the most severely ill patients (Group D) had abnormal corticosteroid excretions, 2 showing reduced and 3 showing probably elevated values. The results obtained in all other patients were within the accepted normal range. The 3 patients with elevated corticosteroid excretions had definitely abnormal responses to the epinephrine test, as did 1 of the patients with reduced urinary corticosteroids. The second patient with low corticosteroids exhibited a questionably abnormal eosinophil response to epinephrine. Of the 6 patients with normal corticosteroid excretions, 2 had a normal response to the epinephrine test, 3 a questionably abnormal, and 1 a definitely abnormal response to epinephrine. No correlation is apparent between the level of the urinary corticosteroids and the degree of clinical severity.

Urinary 17-Ketosteroids. Our normal values for urinary 17-ketosteroids range between 6 and 21 mg. per 24 hours for males (average: 14 mg.) and between 4 and 17 mg. per 24 hours for females (average: 10 mg.). Urinary 17-ketosteroid determinations were carried out on 12 of the 14 patients. The results are listed in Table 4 and are compared with those obtained from the epinephrine test and the urinary corticosteroid studies.

The values obtained from 8 of the 12 patients were below the limits of normal. Four patients, 1 from each of the 4 clinical groups, showed 17-ketosteroid values which fell within the lower range of normal. There appears to be little or no correlation between either the degree of clinical severity or the urinary excretion of corticosteroids and the urinary 17-ketosteroids.

TABLE 4.—URINARY 17-KETOSTEROIDS: COMPARISON WITH EPINEPHRINE TEST, URINARY CORTICOSTEROIDS, AND CLINICAL GROUP

Number	*Sex*	*Urinary 17-ketosteroids, mg. per 24 hr.*	*Corticosteroids, mg. per 24 hr.*	*Epinephrine test, eosinophil change, per cent*	*Clinical group*
		Below normal			
10	F	1.2	0.18	−46	D
6	M	1.6	0.88	−30	D
9	M	1.9	0.85	−44	B
13	M	1.9	——	−66	B
1	M	2.8	1.04	+20	D
3	F	2.9	1.12	− 0.3	D
8	M	2.9	0.88	−43	C
5	M	4.8	1.48	−23	D
		Within normal range			
14	F	5.4	0.39	−72	B
2	F	6.0	0.26	+ 0.4	D
11	M	8.6	0.32	−54	C
4	M	9.9	0.96	− 0.6	A

Sedimentation Rate. We consider the upper limit of normal of the erythrosedimentation rate (Westergren) to be 20 mm. in 1 hour. The sedimentation rate was determined in each of the 14 patients. The results are shown in Table 5 and are compared with the values obtained in the foregoing studies. There is a reasonable parallelism between the sedimentation rate and the degree of clinical severity. The sedimentation rate appears roughly to correlate with the results of the epi-

nephrine test. There seems to be no correlation between either the urinary corticosteroids or 17-ketosteroids and the sedimentation rate.

Comparison of Results With Degree of Clinical Severity. It will be seen from Table 6 that although there is a rough correlation between the gravity of the disease as judged clinically and the degree of abnormality obtained from laboratory studies, the results overlap so much that clear-cut differences are not apparent on the basis of these studies.

Comment. It seems likely that dysfunction of the adrenal cortex is a frequent occurrence among patients with chronic ulcerative colitis, irrespective of clinical severity, but most often accompanying the more advanced forms of the disease. The work of Thorn and his associates and of Laragh and Almy suggests strongly that the abnormalities obtained from the epinephrine test reflect a depletion of adrenal reserve, as the result of long-continued stress. This is most likely a nonspecific phenomenon, in no way

TABLE 5.—SEDIMENTATION RATE: COMPARISON WITH EPINEPHRINE TEST, URINARY 17-KETOSTEROIDS AND CORTICOSTEROIDS, AND CLINICAL GROUP

Number	*Sedimentation rate, mm. in 1 hr.*	*Epinephrine test, eosinophil change, per cent*	*17-ketosteroids, mg. per 24 hr.*	*Corticosteroids, mg. per 24 hr.*	*Clinical group*
10	145	−46	1.2	0.18	D
7	81	−43	—	——	C
5	73	−23	4.8	1.48	D
3	63	− 0.3	2.9	1.12	D
1	58	+20	2.8	1.04	D
6	57	−30	1.6	0.88	D
8	56	−43	2.9	0.88	C
12	55	−65	—	——	A
2	50	+ 0.4	6.0	0.26	D
14	44	−72	5.4	0.39	B
11	42	−54	8.6	0.32	C
9	40	−44	1.9	0.85	B
13	30	−66	1.9	——	B
4	16	− 0.6	9.9	0.96	A

TABLE 6.—CORRELATION OF CLINICAL GROUP WITH LABORATORY STUDIES

Number	*Clinical group*	*Epinephrine test, eosinophil change, per cent*	*17-ketosteroids, mg. per 24 hr.*	*Corticosteroids, mg. per 24 hr.*	*Sedimentation rate, mm. in 1 hr.*
12	A	−65	——	——	55
4	A	− 0.6	9.9	0.96	16
14	B	−72	5.4	0.39	44
9	B	−44	1.9	0.85	40
13	B	−66	1.9	——	30
11	C	−54	8.6	0.32	42
8	C	−43	2.9	0.88	56
7	C	−43	——	——	81
1	D	+20	2.8	1.04	58
10	D	−46	1.2	0.18	145
3	D	− 0.3	2.9	1.12	63
6	D	−30	1.6	0.88	57
5	D	−23	4.8	1.48	73
2	D	+ 0.4	6.0	0.26	50

peculiar to chronic ulcerative colitis. Similar findings may be expected in other severe protracted diseases.

It is difficult to reconcile the urinary corticosteroid excretions with the results of the epinephrine test, since abnormal responses to epinephrine were found in the face of diminished, normal and elevated urinary corticosteroids. It is tempting to speculate that in the cases of elevated corticosteroids, the adrenals were already responding maximally, and were unable to react additionally to any further excitation, thus accounting for the impaired eosinophil response.

In the 2 instances in which diminished excretion of corticosteroids accompanied abnormal results of epinephrine tests the adrenal cortices may have been exhausted beyond their capability to respond to further stimulus. It is possible that determinations of the corticosteroid excretions following the epinephrine test would have given further information as to the functional state of the adrenal cortex in these cases.

One may be tempted to dismiss the epinephrine test as eliciting a response which does not involve the adrenal cortex. Although this remains a possibility, such hardly would seem to be the case in view of the work of Laragh and Almy.

The determination of urinary 17-ketosteroids was of little value aside from demonstrating the decline in these values often seen in any debilitating condition.

Summary. An attempt was made to assess adrenal cortical reserve function in a group of 14 patients suffering from chronic ulcerative colitis of varying degrees of severity. As determined by the epinephrine test, questionably impaired adrenal function was found in 5 of 14 patients and definite dysfunction was evident in another 5. The results of this procedure roughly paralleled the degree of clinical severity. There was no correlation between the results of the epinephrine test and urinary corticosteroid excretion as measured prior to the test; abnormal responses to epinephrine were accompanied by diminished, normal and elevated corticosteroid values. Low normal or abnormally diminished urinary 17-ketosteroids were found in all patients studied, with no apparent relation between these values and the urinary corticosteroids or the results of the epinephrine test. The sedimentation rate was found roughly to parallel the results of the epinephrine test and the degree of clinical severity. The findings are briefly discussed. It is felt that the evidences of impaired adrenal cortical function represent a depletion of cortico-adrenal reserve which is not specific for chronic ulcerative colitis and which is the result of long-continued stress.

Conclusions. As judged by the response to the epinephrine test of Laragh and Almy impairment of the reserve function of the adrenal cortex is not infrequently an accompaniment of chronic ulcerative colitis.

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THE EFFECT OF VITAMIN E ADMINISTRATION UPON DIABETES MELLITUS*

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The statement has been made that all diabetics over 25 years of age show a marked decrease of insulin requirements within 2 months of the start of vitamin E administration, and that this therapy in no case aggravates the condition[2,3,10,11]. This finding, if verified, would bring about a revolution in the current clinical management of diabetes mellitus. Physicians who are interested in the treatment of diabetes are naturally anxious to utilize all proven new developments in therapy. We report our observations in 27 patients so treated.

Newer therapies in diabetes mellitus require an evaluation based upon a thorough understanding of the natural history of development of this clinical syndrome. One must recognize that spontaneous remissions do occur in clinical diabetes. Numerous instances of these have been reported in the literature[1,5,6,7,8]. In addition, there is a tendency for the glycosuria of newly discovered diabetes to ameliorate after therapy has been started. One frequently sees patients who require the administration of insulin to control their glycosuria when the disease is first discovered, but who in a variable time may remain aglycosuric after insulin therapy is stopped. The proper selection of test patients in assaying new therapeutic procedures is of paramount importance. Patients who have been under treatment for diabetes mellitus for more than 2 years, and whose behavior pattern has been established, are preferable to patients with newly discovered diabetes, for the above mentioned reasons.

Material and Method. To this end, 27 patients with diabetes mellitus were selected for vitamin E therapy. These patients had been under observation in the metabolism clinic of The Mount Sinai Hospital from 3 to 25 years, and had been observed at least 5 times annually during this period. Their insulin requirements, dietary pattern and indiscretions, susceptibility to hypoglycemia, as well as their glycosurias, were a matter of hospital record.

Of these patients, 26 required insulin to control the symptoms of their diabetes at the onset of the test period. It is to be noted that the patients selected were those who had some degree of glycosuria. This was done in order to have a quick index of improving carbohydrate tolerance. Thus, if the vitamin E administration proved to be efficacious, the glycosuria would decrease. Insulin dosage could then be reduced before hypoglycemic reactions were produced. It was felt that the induction of hypoglycemia would be detrimental to this group of patients.

* We are indebted to Hoffman LaRoche for supplies of α-tocopherol acetate.

TABLE 1.—CLINICAL DATA ON EFFECT OF VITAMIN E UPON DIABETES AS OBSERVED IN 27 CASES

Pt.	Clinic No.	Age	Sex	Years of Known Diabetes	Range of Patient's Dosages of Insulin	Dose of 100 mg. of Vitamin E	Weeks of Administration	Weight in Pounds at Start	Weight in Pounds at End	Glycosuria at Start 1*	2	3	4	Glycosuria at End 1	2	3	4	Dose of Insulin at Start	Dose of Insulin at End	Vascular Changes
1	40–4132	47	M	10	40–60 u	Synthetic	13	158	158	0	0	4+	3+	4+	4+	3+	3+	60 u	60 u	
2	47–7536	46	F	9	12–40 u	"	11	243	246	Tr	2+	0	0	Tr	0	0	0	40 u	40 u	Hypertension, E.C.G. changes
3	39–836	50	F	5	40–80 u	"	9	160	162½	4+	4+	4+	4+	0	4+	4+	4+	50 u	50 u	Retinopathy
4	33–7752	61	F	22	25–50 u	"	11	161½	162	2+	Tr	Tr	3+	0	4+	+	+	50 u	40 u	Hypertension, E.C.G. changes
5	34–15326	53	F	4	25–35 u	"	6	160½	161¾	0	+	+	3+	0	Tr	Tr	Tr	35 u	35 u	Hypertension, E.C.G. changes
6	33–18252	59	F	11	40–80 u	"	6	226	228	4+	4+	4+	4+	Tr	0	0	0	40 u	50 u	Hypertension, retinopathy
7	34–18204	59	F	9	0–40 u	"	12	178½	181	+	(24 hr. spec.)			+	4+	3+	4+	40 u	60 u	
8	33–6630	63	F	11	0–15 u	"	11	209½	211½	+	+	+	+	3+	4+	4+	4+	15 u	15 u	E.C.G. changes
9	40–1103	22½	F	8	70–80 u	"	12	213½	216¼	Tr	Tr	Tr	Tr	0	0	0	0	80 u	80 u	
10	33–15579	54	F	5	15–40 u	"	3	239	242	Tr	4+	0	Tr	3+	3+	3+	3+	40 u	40 u	Hypertension, E.C.G. changes
11	40–1338	67	M	25	80–200 u	"	7	156½	145	4+	4+	4+	4+	4+	4+	4+	4+	95 u	245 u	Cerebral arteriosclerosis
12	33–13616	69	F	19	0–80 u	Natural	3	152½	152½	+	+	0	+	0	0	0	0	80 u	80 u	Coronary arteriosclerosis (pt. died)
13	33–24221	65	F	16	0–40 u	"	9	140	139	Tr	3+	3+	4+	4+	4+	Tr	Tr	40 u	50 u	
14	40–3734	67	F	19	0	"	11	182	183	4+	(24 hr. spec.)			0	0	0	0	0	20 u	Hypertension, E.C.G. changes
15	40–5350	54	F	9	20–50 u	"	4	190	187½	3+	3+	Tr	0	2+	0	0	0	50 u	55 u	E.C.G. changes
16	40–7007	59	F	9	0–15 u	"	10	125	125	3+	(24 hr. spec.)			2+	(24 hr. spec.)			10 u	12 u	Coronary arteriosclerosis
17	44–2381	19½	M	5	50–110 u	"	11	151	151	2+	2+	2+	4+	2+	0	0	4+	110 u	100 u	
18	47–1944	46	F	3	30–60 u	"	7	119	119¾	0	0	0	0	4+	3+	4+	3+	62 u	65 u	
19	36–8900	61	M	4	10–70 u	"	12	170	164	4+	4+	4+	4+	3+	4+	1+	4+	75 u	85 u	Hypertension, E.C.G. changes, periph. vasc. disease
20	33–1841	43	M	17	60–70 u	"	7	126	122¼	4+	(24 hr. spec.)			0	(24 hr. spec.)			68 u	57 u	Retinopathy
21	37–7121	41	F	9	60–100 u	"	12	136	138	2+	4+	4+	4+	4+	4+	4+	4+	90 u	100 u	E.C.G. changes
22	36–9675	45	F	17	30 u	Synthetic	13	108	107½	Tr	+	+	0	+	0	+	2+	30 u	30 u	Kimmelstiel-Wilson Syndrome
23	46–4199	44	F	12	30–50 u	"	13	127	124	Tr	+	+	0	0	+	+	Tr	36 u	30 u	Retinopathy
24	33–4140	62	F	19	15–25 u	Natural	11	160	159½	0	3+	+	4+	0	Tr	4+	Tr	25 u	25 u	Hypertension, E.C.G. changes
25	33–2750	65	F	23	30–100 u	"	13	137¼	137	4+	4+	4+	4+	Tr	2+	4+	Tr	85 u	85 u	Hypertension, retinopathy, cataracts
26	33–927	69	F	17	50–70 u	"	7	156	157	4+	4+	4+	4+	4+	4+	3+	3+	60 u	60 u	Hypertension, E.C.G. changes, retinopathy
27	41–3519	27	M	8	35–50 u	"	4	148½	147½	4+	0	+	+	0	4+	4+	0	50 u	35 u	

* See text for periods designated by *1, 2, 3, 4*.

The original claims for the beneficial effects of vitamin E were made in the arteriosclerotic group[9,12]. This series was selected to include many patients with arteriosclerosis, as indicated in Table 1.

The prescribed diets were unchanged during the period of observation. Thirteen of these patients received the synthetic alpha tocopherol. The remainder received the natural distilled mixed tocopherols. The dosage was 400 mg. daily (100 mg. 4 times a day). The data on the individual patients are indicated in Table 1.

The glycosurias were determined on each patient before the administration of vitamin E and at intervals during the period of the test. Fractional urines were collected. Urine collection 1 was for the overnight period; urine collection 2 was from breakfast to luncheon; urine collection 3 was from luncheon to supper; and urine collection 4 was from supper to bedtime. The diurnal variations in glycosurias are represented for the first and last day of the test period. There were no significant variations observed during the entire time of the test.

Results. Patient 14 developed such marked symptoms that insulin administration had to be started during the course of this test. It is to be noted that no insulin had to be administered in the previous 19 years of her care. Patient 20, whose glycosuria shows an apparent improvement, and whose insulin requirements were decreased 11 units, had a record of similar spontaneous fluctuations in his diabetes several times in the past years of his attendance at the metabolic clinic. Patient 27 discontinued the vitamin E of his own accord, because of subjective discomforts. Many of the patients complained of gastrointestinal disturbances, which interfered with their appetite; and some reported diminution in food intake. Patient 12 died 3 weeks after the test was started. The cause of death was acute coronary occlusion. Patients 10 and 11 had to be hospitalized for control of their diabetes during this observation, and vitamin E was discontinued upon their admission to the ward. Patient 11 developed marked ketosis, loss of weight, and polyuria.

Conclusions. The administration of vitamin E, both natural and synthetic in dosage of 400 mg. daily, to known diabetics, whose previous fluctuations were well known, exerted no beneficial effect upon the insulin requirement or glycosuria.

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BLOOD FACTOR IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS
1. DETERMINATION OF GAMMA GLOBULIN AS SPECIFIC PLASMA FRACTION

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Independent investigations[1,2,3,4] have shown that the plasma of patients acutely ill with disseminated lupus erythematosus contains a factor which induces the formation of rosettes of leukocytes and a characteristic inclusion-containing cell, the lupus erythematosus (LE) cell, when mixed with normal bone marrow preparations.

This study was designed to identify the fraction of the LE plasma responsible for inducing the phenomenon.

Method. After initial studies had revealed that both serum and plasma were capable of producing the phenomenon, plasma was used exclusively in order to simplify and standardize the procedure. Plasma from 5 patients with acute disseminated lupus erythematosus was used. Each of the 5 plasma samples consistently induced both phases of the phagocytic phenomenon when mixed as whole plasma with normal bone marrow preparations. Fractions of the plasma samples were then prepared, and were added individually to normal bone marrow preparations.

Before fractionation, the samples of lupus plasma (LEP) were dialyzed. This did not change their ability to induce the phenomenon. After dialysis each plasma was fractioned by the Tiselius electrophoretic technique, using phosphate buffer pH 7.8, ionic strength 0.16μ. Fractions of LEP and their approximate quantities prepared in this way were: 1, Albumin, (3.5 mg.); 2, Albumin, alpha and beta globulin (12 mg.); 3, Gamma globulin (2 mg.); and 4, Gamma globulin and fibrinogen (4.8 mg.). The fractions were added to normal* bone marrow preparations by the method previously reported for whole plasma[2,3]. Each mixture was studied for rosettes of leukocytes and LE cells, to determine which fraction of the LEP carried the responsible factor.

Results. Tables 1 and 2 summarize the plasma fractions producing leukocytic clumping and LE cells, respectively. It is emphasized that both of these findings represent phases of the same phagocytic phenomenon. The division of the phenomenon into rosettes of leukocytes and the LE cell is made to permit wider investigation, for it may be that their presence indicates important plasma differences.

The whole plasma induced both parts of the phenomenon, and produced a more clearcut alteration than any one fraction. The gamma globulin induced the formation of rosettes of leukocytes (Fig. 1 A) in all 5 cases, and the LE cell (Fig. 1 B-E) in 4 of the 5. The combined gamma and fibrinogen induced both parts of the phenomenon in 1 case, but was not tested in the other 4 when it was discovered that serum induced the phagocytic phenomenon as effectively as plasma. The

* Normal = the non-lupus bone marrow material obtained routinely for the study of anemias, etc., in the special hematology laboratory.

albumin and the albumin plus alpha and beta globulin portions of the LEP failed to induce either stage of the phagocytic phenomenon.

In 3 cases the whole plasma and the separate fractions were tested on more than one normal marrow. In 1 case the gamma globulin, when used in half the usual concentration, failed to induce either phenomenon; on using a more concentrated gamma globulin preparation the leukocytic clumping but not the LE cell appeared. The variation in marked variations from the normal pattern. The gamma globulin and the α^2globulin were elevated above the maximum normal and the albumin concentration was decreased.

Comment. The results of these observations indicate that the factor of LEP responsible for inducing leukocytic clumping and the LE cells in normal bone marrow preparations is related to the gamma globulin fraction. That it is not the gamma globulin alone, but a substance associated with it that is

TABLE 1.—PLASMA FRACTIONS PRODUCING LEUKOCYTIC CLUMPING

Patient	*Whole Plasma*	*Albumin*	*Albumin-Alpha & Beta Globulin*	*Gamma Globulin*	*Gamma Globulin & Fibrinogen*
Mrs. V.	+	0	0	+	+
Miss S.	+	0	0	+	
Miss H.	+	0	0	+	
Mrs. L.	+	0		+	
Mrs. S.	+	0		+	

TABLE 2.—PLASMA FRACTIONS PRODUCING THE LE CELL

Patient	*Whole Plasma*	*Albumin*	*Albumin-Alpha & Beta Globulin*	*Gamma Globulin*	*Gamma Globulin & Fibrinogen*
Mrs. V.	+	0	0	0	+
Mrs. L.	+	0	0	+	
Miss S.	+	0	0	+	
Miss H.	+	0	0	+	
Mrs. S.	+	0		+	

the ability of gamma globulin to induce the phenomenon was found to be related to the concentration of the gamma globulin in milligrams, rather than to its percentage of the patient's whole plasma. A diluted gamma globulin fraction, for example, induced only the leukocytic rosettes, while a more concentrated portion produced both the rosettes and the LE cell.

Though it is not the purpose of this paper to report the Tiselius patterns, it is noteworthy that the plasma protein pattern of the 5 cases of acute disseminated lupus erythematosus showed responsible for the phagocytic phenomenon has been previously determined in the clinical reports[2,3] in which many conditions with a striking increase of the gamma globulin were found to lack the ability to induce the LE phenomenon.

A further correlation exists between these *in vitro* studies and the variations in the course of the disease. Plasma from patients entering into remissions first loses its property of producing LE cells, the leukocytic clumping being the last part of the LE phenomenon to disappear with improvement in the pa-

tient's condition. However, both phases of the LE phenomenon disappear when the remission is more complete. This was noted in 2 patients. During a relapse, the plasma of 1 of these patients again was found to contain the factor

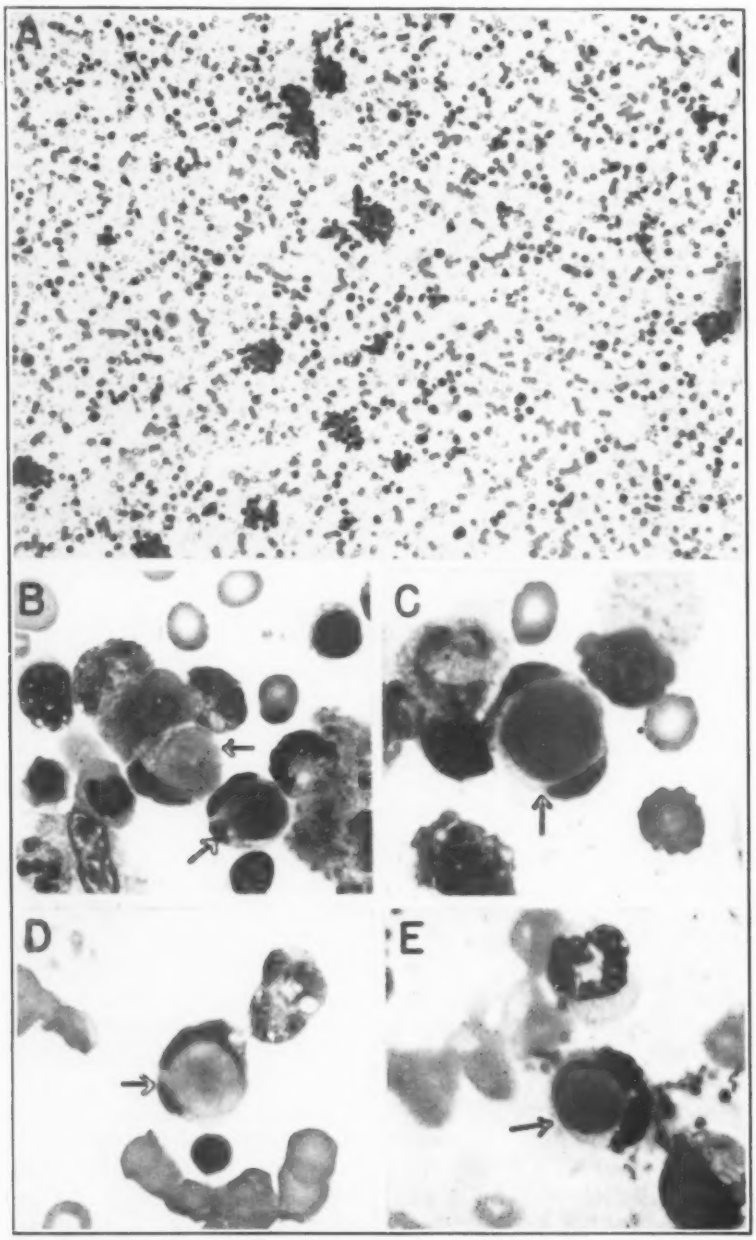

FIG. 1 A.—Clumping of leukocytes induced in normal bone marrow by LE gamma globulin. Note rosette formation (x 100).

FIG. 1 B to E.—Arrows point to LE cells induced in normal bone marrow by LE gamma globulin. Note large, round, homogenized inclusions (x 700).

which induced the complete LE phenomenon in normal bone marrow preparations. The *in vitro* variations noted with different concentrations of gamma globulin compared favorably with the waxing and waning of the clinical aspects of the disease.

This suggests that the LE cell is the last step in a 2 stage phagocytic phenomenon which is first observed as clumping of leukocytes around a blue staining mass. By some process of phagocytosis the mass is engulfed to form the homogenized, well circumscribed inclusion body seen in the LE cell. The engulfed material is thought at present to be of megakaryocytic or platelet origin, though it may well be collections of amorphous protein. Further investigations on this material are being carried out.

While the bone marrow findings are a striking feature and represent a phenomenon which is poorly understood, it is important to emphasize that the bone marrow alterations are simply indicators of the presence of the LE factor. Since the detection of the LE factor depends entirely upon its ability to induce the LE phenomenon in normal bone marrow preparations, the bone marrow alterations make the only available endpoint for assaying the presence of LE factor in suspected plasma. It is not inconceivable that future investigations may develop simpler methods for indicating the presence of I E factor, and the LE plasma-normal bone marrow procedure be discarded entirely.

Summary. To determine what fraction of LEP contains the factor responsible for inducing the LE phenomenon in normal bone marrow—LEP mixtures, whole plasma from 5 patients with acute disseminated lupus erythematosus was separated into 4 fractions by the Tiselius electrophoretic technique.

Each fraction was added separately to different normal bone marrow preparations. The mixtures were studied for the 2 phases of the LE phenomenon—rosettes of clumped leukocytes and, or, LE cells.

Only the gamma globulin fraction produced these 2 stages of the phenomenon of phagocytosis. The albumin fraction and the combined albumin, alpha and beta fraction, failed to induce the phenomenon. A fourth fraction, containing both gamma globulin and fibrinogen, induced the phenomenon, but the fibrinogen was eliminated as a possibility when it was found that serum was as effective as plasma in producing the bone marrow change.

The LE factor was found to disappear from the blood during remissions in 2 patients with acute disseminated lupus erythematosus, but was found again during the relapse in 1 patient. A similar change was noted when LE gamma globulin was quantitatively reduced *in vitro*. With moderate dilution of the gamma globulin the LE cell disappeared from the preparations, but the leukocytic clumping persisted. With greater dilution of the gamma globulin both phases of the LE phenomenon were absent.

Conclusions. 1. The gamma globulin of acute disseminated lupus erythematosus contains the factor which is responsible for inducing rosettes of leukocytes and formation of the LE cell in mixtures of normal bone marrow preparations and LE plasma.

2. The LE factor disappears from the blood during remissions, and reappears during relapses in patients with acute disseminated lupus erythematosus.

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OBSERVATIONS ON THE GLUCOSE TOLERANCE TEST IN PAGET'S DISEASE (Osteitis Deformans)

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Disturbances in glucose tolerance in Paget's disease (osteitis deformans) have been described by several workers[2,4,8,11] and have been offered as evidence that this disease is basically an endocrine or diffuse metabolic disturbance[2,11]. Althausen[1] noted an impaired tolerance to both glucose and galactose in Paget's disease and attributed the high post-absorptive blood levels of these sugars to an accelerated rate of intestinal absorption.

The demonstration by the Coris and Schmidt[5] of the essential role of phosphatase in the production of blood glucose by its promotion of the dephosphorylation of glucose phosphates suggested an explanation for the reported altered carbohydrate metabolism in Paget's disease, a condition characterized by an elevated serum alkaline phosphatase.

The work here reported was designed to re-study the problem of glucose tolerance in Paget's disease and to test the hypothesis that elevated serum alkaline phosphatase in patients and in experimental animals would depress tolerance to glucose probably by accelerating the production of blood glucose from glucose-phosphates.

Clinical Studies. **Methods.** Patients with unequivocal osteitis deformans were accepted for this study if they were in reasonably good health, aside from their bone disease, and presented no clinical or laboratory evidence of diabetes mellitus or of significant liver dysfunction. Ten patients who met these criteria were subjected to intravenous glucose tolerance tests, and 7 were retested by the oral method. In several patients the tests were repeated. A battery of liver function tests and estimations of the serum calcium, phosphorus and phosphatase, cholesterol and cholesterol esters, blood urea nitrogen, and basal metabolism were carried out in the majority of patients, to ascertain more adequately their suitability for inclusion in the study. A control group was selected from healthy volunteers whose ages were comparable to the patients with Paget's disease, and was similarly studied with oral and intravenous glucose tolerance tests. Selection of these subjects was based on their freedom from signs or symptoms of disease, a negative personal and family history of diabetes mellitus, absence of hepatomegaly and evidence of normal liver function by laboratory studies.

The single 100 gm. dose oral glucose tolerance test was used, and post-absorptive blood sugars were determined 30, 60, 90, 120 and 180 minutes thereafter. The intravenous glucose tolerance test was that described by Soskin[16]. One-third gram of dextrose per kg. of body weight in a 50% aqueous solution was injected intravenously within a period of 3 to 5 minutes, and blood sugar estimations were made 15, 30, 60, 75, 90 and 120 minutes thereafter. Microchemical blood sugar estimations using capillary blood were determined either by the Somogyi-Shaffer-Hartman[14a,b,c] method or by the technique of Lauber and Mattice[9]. The unpredictable influence of non-glucose reducing substances was thus eliminated[12]. Normal values for true blood sugar by these methods are 60 to 90 mg. per 100 cc. of blood. Serum alkaline phosphatase was determined by Bodansky's method[3] with one exception (Case C).* Fructose was determined by Roe's method[13].

All tests were done in the morning after an overnight fast. Though the influence of the

* The phenophthalein method of Huggins was used in this case (J. Biol. Chem., **159**, 399, 1945). Normal values are 3 to 15 units.

TABLE 1.—ORAL GLUCOSE TOLERANCE TESTS. VALUES ARE TABULATED AS MILLIGRAMS ABOVE (+) OR BELOW (−) THE INITIAL FASTING BLOOD SUGAR VALUES. (ABSOLUTE FASTING BLOOD SUGAR VALUES ARE SHOWN IN PARENTHESES.)

(*A*) CASES OF PAGET'S DISEASE

Pt.	*Age*	*Sex*	*F*	*Minutes After Giving Glucose* 30	60	90	120	150	180
B	56	M	(78)	+56	+70	+59	+46	+50	+60
C	52	*F*	(53)	+74	+124	+130	+108	+99	+92
D	55	F	(68)	+88	+51	+44	+35	−18	−42
E	72	M	(85)	+46	+70	+30	+9	−17	−25
F	67	M	(71)	+29	+36	+20	+53	+85	+117
G	50	M	(86)	+34	+60	+28	+20	+4	—
K	66	F	(86)	+97	+58	—	+22	0	−18

	Mean	*Range*
90 min.	51.8	+20 to +130
120 min.	41.9	+9 to +108
150 min.	29.0	−18 to +99

(*B*) NORMAL CONTROLS

5	71	M	(56)	+62	+58	+38	+30	+26
6	74	M	(64)	+22	+58	+74	+68	+44
9	74	M	(64)	+100	+82	+82	+48	+38
15	60	M	(88)	+48	+108	+94	+44	+34
3	66	M	(66)	+34	+68	+80	+68	+48
10	83	M	(90)	+76	+182	+168	+142	+76
12	80	M	(72)	+118	+150	+198	+178	+158
13	80	M	(72)	—	+118	+114	+86	+36

	Mean	*Range*
90 min.	106	+38 to +198
120 min.	83	+30 to +178
150 min.	57.5	+26 to +158

"t" values— 90 min.—2.12
120 min.—2.80
150 min.—1.22

preliminary diet on the glucose tolerance test was recognized[7], the carbohydrate intake in the period preceding the test could not be rigidly supervised because most of the subjects were ambulatory out-patients. They were all, however, eating a normal diet according to generally accepted standards.

Results. The data for the comparison of the oral glucose tolerance test in 7 patients exhibiting osteitis deformans with 8 normal controls selected from a similar age group is listed in Table 1. The mean values and the range are graphically depicted in Fig. 1A. It is necessary in comparative studies of glucose tolerance tests to compare curves initiating from identical fasting values. All curves are graphed and plotted, therefore, as the percentage change from a zero fasting value, rather than plotted as absolute blood sugar values beginning at varying fasting levels. One would be justified, using generally accepted standards as criteria, in considering the tolerance for glucose to be moderately depressed in the patients with Paget's disease. The mean curve for the 7 subjects failed to return to the fasting level within 3 hours, the so-called "delayed return curve". Osteitis deformans is a disease of middle life and old age. Several investigators[6] have shown a gradual low-

ering of carbohydrate tolerance with advancing age as measured by the oral glucose tolerance test. When we compared the tests in the group of Paget's disease whose mean age was 60 years with the control group with a mean age of 74 years, the observed differences might well be explained on the basis of age alone; *i.e.*, the controls showed a more abnormal glucose tolerance than the Paget group. The difference was not statistically significant when analyzed by a modification of Fisher's "t"[10] applied to the blood sugar values in the 2 groups at 90 and 150 minutes (see Table 1). The "t" of 2.80 at 120 minutes, which does show a statistically significant difference, can be accounted for by the grossly abnormal test of Subject 12.

Importance is sometimes attached to the maximum height attained by the peak of a glucose tolerance test. For the Paget's patients the mean peak was 85 mg. above the fasting level (range 58 to 130 mg.) and for the controls 132 mg. (range 62 to 198 mg.). Here again the control group showed a greater variation from normal than the subjects with osteitis deformans.

The results of the intravenous glucose tolerance tests in 10 patients with osteitis deformans and in 15 normal elderly subjects are presented in Table 2 and Fig. 1B. The mean curves for the 2 groups closely approximate each other. Statistical treatment of the values at 60, 75, and 90 minutes shows no significant differences (see Table 2). The patients with Paget's disease disposed of the intravenously administered glucose at the same rate as normal subjects.

When the patients with osteitis deformans who showed elevated serum alkaline phosphatase levels were compared with those with normal or only slightly elevated serum alkaline phosphatase, and hence probably in an inactive phase of the disease, no significant difference was found. Several patients with high serum phosphatase due to

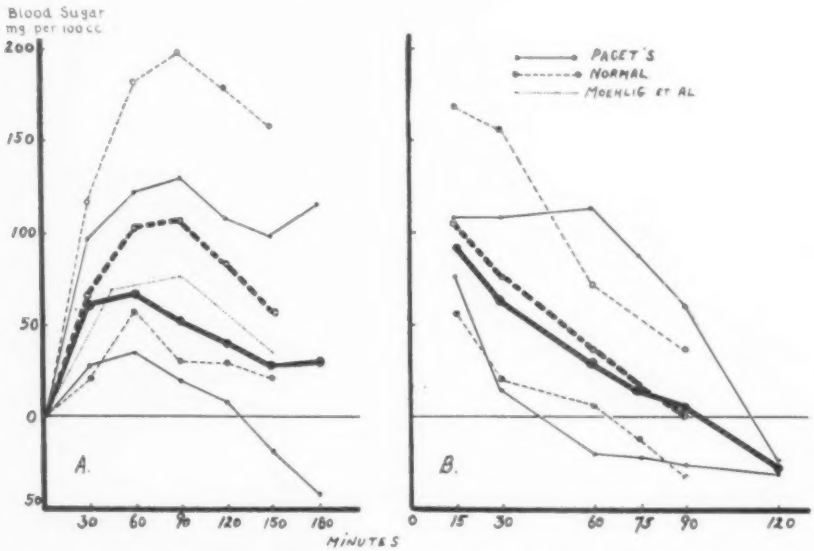

FIG. 1. Oral (A) and intravenous (B) glucose tolerance tests in Paget's disease and in normal controls. The heavy lines are the means, and the thin lines the range.

bone metastasis from prostatic carcinoma and one patient with neurofibromatosis and hyperphosphatasemia were subjected to oral and intravenous glucose tolerance tests to investigate the tentative hypothesis that elevated blood phosphatase *per se* should produce prolonged and elevated curves. The results, however, were within normal limits. Where the percentage of abnormality of the intravenous glucose tolerance test, calculated as the number of milligrams above the initial fasting value 60 minutes after the glucose was injected, was compared with the elevation of serum alkaline phosphatase in patients with Paget's disease or osseous metastasis from prostatic carcinoma, no correlation was found.

EXPERIMENTAL STUDIES. A direct experimental approach to the study of

TABLE 2.—INTRAVENOUS GLUCOSE TOLERANCE TESTS. VALUES ARE TABULATED AS IN TABLE 1.

(*A*) CASES OF PAGET'S DISEASE

Pt.	*Age*	*Sex*	*Ser. Alk. P'tase B.U./100 cc.*	*F*	*Minutes After Giving Glucose*						
					15	*30*	*60*	*75*	*90*	*120*	*180*
A	65	M	42.7	(78)	—	+55	+31	+16	+1	−5	—
B	56	M	34.3	(67)	+109	+84	+65	+43	+26	−13	−26
C	52	F	280*	(69)	+106	+67	+12	−8	−26	−20	−29
D	55	F	5.9	(60)	—	+15	+8	+1	−2	−4	—
E	72	M	3.6	(113)	—	+108	+42	+17	+13	−21	−25
F	67	M	6.6	(91)	—	+50	−20	−23	−17	−6	—
G	50	M	11.0	(74)	+82	+42	+14	+2	−2	0	—
H	72	F	24.2	(102)	—	+71	+29	+15	+1	−25	−31
I	80	F	12.1	(79)	—	+85	+114	+87	+60	+34	—
J	64	F	4.8	(222)	+76	+64	+4	+2	+1	+8	—

* Phenolphthalein Method

	Mean	*Range*
60 min.	+29.9	−20 to +114
75 min.	+15.2	−23 to +87
90 min.	+5.5	−26 to +60

(*B*) NORMAL CONTROLS

1	58	M	(60)	+142	+156	+56	+28	+8
2	62	M	(72)	+108	+84	+40	+14	−10
3	66	M	(74)	+70	+54	+12	+2	0
4	73	M	(48)	+94	+74	+48	+16	+4
5	71	M	(88)	+128	+32	+10	−24	−32
6	74	M	(56)	+56	+40	+6	+4	—
7	73	M	(84)	+102	+70	+42	—	—
8	70	M	(86)	+168	+108	+70	+30	+18
9	74	M	(100)	+100	+22	+8	+4	−4
10	83	M	(106)	+116	+74	+66	+42	+8
11	84	M	(56)	+88	+60	+10	+4	0
12	80	M	(60)	+112	+86	+62	+48	+36
13	80	M	(90)	—	+152	+66	+40	+8
14	82	M	(74)	+114	+74	+42	+30	−2
15	60	M	(72)	+82	+48	+14	−12	−18

	Mean	*Range*
60 min.	+36.8	+6 to +70
75 min.	+16.1	−12 to +48
90 min.	+1.1	−32 to +36

"t" values—60 min.—0.55
75 min.—0.09
90 min.—0.55

carbohydrate metabolism in osteitis deformans is not possible. The disease cannot be reproduced in laboratory animals. We have attempted to explore a single facet of the problem by elevating the serum alkaline phosphatase of dogs with intravenous injections of phosphatase solutions.

These solutions maintained their potency indefinitely at ice-box temperatures. They were well tolerated by dogs when injected intravenously, and, though unsterile, caused no alteration in rectal temperature throughout the experiments.

Mongrel dogs, supplied by the Chicago pound and fed hospital scraps supplemented with meat, were the experimental subjects.

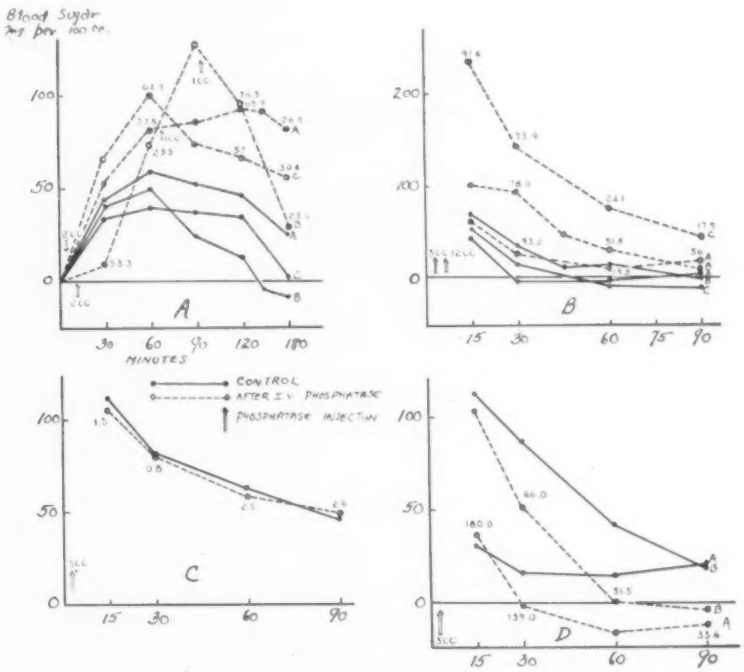

FIG. 2. A. Oral glucose tolerance tests in 3 dogs (A, B, C) before and after the intravenous injection of phosphatase solution I. B. Intravenous glucose tolerance tests in 3 dogs (A, B, C) before and after the intravenous injection of phosphatase solution II. C. Intravenous glucose tolerance tests in a dog before and after the intravenous injection of heat-inactivated phosphatase solution I. D. Intravenous glucose tolerance tests in 2 dogs (A, B) before and after the intravenous injection of phosphatase solution III. Numerals on the glucose curves are Bodansky Units of phosphatase per 100 cc. blood.

Methods. The phosphatase solutions were obtained from 3 sources. Solution I was a preparation from the small intestinal mucosa of dogs kindly supplied us in liberal quantities by Dr. George Gomori of the University of Chicago. The potency of this solution was 200 Bodansky units per cc. Solution II was prepared in our laboratory under Gomori's instructions and contained 175 B.U. per cc. Solution III was similarly prepared from cattle intestines and assayed at 160 B.U. per cc.

Oral glucose tolerance tests were performed by administering 1.75 gm. of glucose per kg. of body weight in a 20% solution via stomach tube. When the intravenous test was done, 0.5 gm. of glucose per kg. in a 50% solution was injected intravenously within 3 to 4 minutes. The phosphatase solutions were injected intravenously during the experiments at an approximate rate of 1 cc. per min. at intervals necessary to maintain a sustained elevation of the serum alkaline phosphatase.

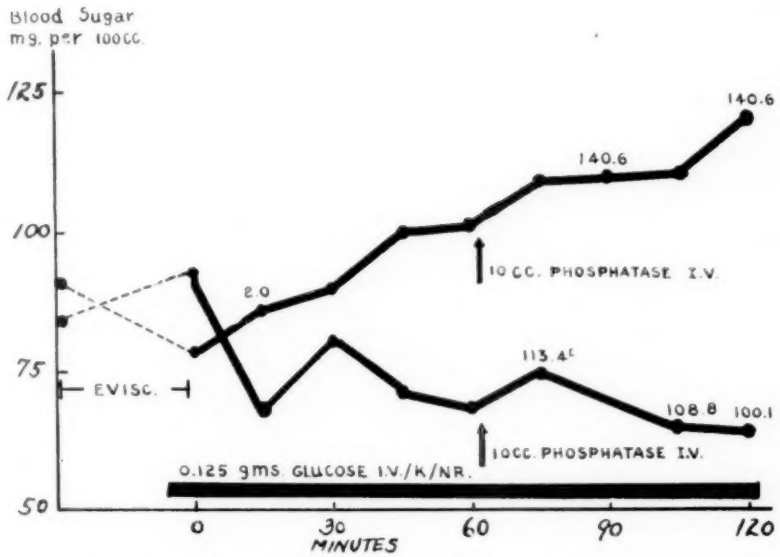

FIG. 3. The effect of intravenously injected phosphatase on the peripheral uptake of glucose in 2 eviscerated dogs. Numerals on the glucose curves are Bodansky Units of phosphatase per 100 cc. of blood.

TABLE 3. THE INFLUENCE OF THE INTRAVENOUS INJECTION OF PHOSPHATASE SOLUTION ON BLOOD SUGAR

	Dog 9792	Weight 6.5 Kg.	16 April 1947
Time	*Blood Sugar (Mg./100 cc.)*	*Ser. Alk. Phosphatase B.U./100 cc.*	*Procedure*
10:05	75	—	
10:20	87	3.7	
10:36	92	—	
10:50	89	—	
11:05	—	—	Phosphatase Sol. I—3 cc. I-V
11:20	95	—	
11:35	101	116.4	
11:50	101	—	
12:05	103	52.4	
	Dog 9697	Weight 7.5 Kg.	21 May 1947
9:35	90	—	
9:50	75	—	
10:05	72	—	
10:20	80	—	
10:35	73	2.0	
10:43	—	—	Phosphatase Sol. II—14 cc. I-V
10:58	93	62.6	
11:25	92	5.1	
11:27	—	—	Phosphatase Sol. II—6 cc. I-V
11:42	92	5.1	
11:57	72	4.8	

Results. The intravenous injection of a phosphatase solution produced a marked and sustained elevation of the serum alkaline phosphatase. The serum phosphatase activity after the injection of a single dose of from 3 to 10 cc. was unpredictable, varying from 37 to 180 Bodansky units per 100 cc., and gradually falling to values of from 30 down to 5.6 units per 100 cc. (see Fig. 2, A, B and D) in from 1½ to 3 hours. A second injection of phosphatase 60 to 90 minutes after the first pushed the level well over 100 B.U. per 100 cc. The most sustained high values were obtained in the eviscerated dogs receiving 10 cc. of Solution II (see Fig. 3).

EXPERIMENTS AND RESULTS. A. *Control Experiments.* The influence of the phosphatase solutions *per se* on blood sugar was determined in 2 studies. A slight rise occurred (see Protocol in Table 3.). The average glucose value

TABLE 4.—ORAL AND INTRAVENOUS FRUCTOSE TOLERANCE TESTS BEFORE AND AFTER THE INTRAVENOUS INJECTION OF PHOSPHATASE SOLUTION

		Dog 0380	Weight 6.5 Kg.	14 April 1947
Time	*Blood Glucose (Mg./100 cc.)*	*Blood Fructose*	*Ser. Alk. P'tase B.U./100 cc.*	*Procedure*
9:00	92	2	—	
9:07	—	—	—	11.4 gm. fructose via stomach tube
9:22	97	2	—	
9:37	96	13	—	
10:07	110	40	—	
10:37	128	20	—	
11:07	117	8	—	
12:00	127	3	2.2	
12:35	—	—	—	Phosphatase Sol. I, 3 cc. intravenously
				11.4 gm. fructose via stomach tube
12:52	173	5	85.2	
1:07	174	6	—	
1:15	—	—	—	Phosphatase: 2 cc. intravenously
1:22	169	8	131.0	
1:37	158	8	—	
2:07	117	4	62.1	Phosphatase: 1 cc. intravenously
2:37	110	11	23.3	
		Dog 9714	Weight 5.2 Kg.	27 May 1947
8:40	91	1	—	
8:47	—	—	—	2.6 gm. Fructose intravenously
9:02	127	14	—	
9:17	103	7	—	
9:47	104	6	—	
10:17	108	4	—	
10:40	122	3	2.0	
10:48	—	—	—	Phosphatase Sol. I, 3 cc. intravenously
10:53	—	—	—	2.6 gm. Fructose intravenously
11:08	142	23	—	
11:23	118	11	169.0	
11:53	139	6	—	
12:23	127	6	33.2	

before the phosphatase was injected was 82 mg.; and after phosphatase, 94 mg.

Glucose tolerance tests were performed by the oral and intravenous routes several times during a period of 1 or 2 weeks upon the same animals to ascertain the normal variation of repeated tests. In every instance little significant variation occurred.

B. *The Influence of Hyperphosphatasemia on the Oral Glucose Tolerance Test.* A few days after performing an oral glucose tolerance test, the test was repeated on the same animal following intravenous injections of phosphatase. The results of 3 such experiments shown in Fig. 2A demonstrate a reduction of tolerance to glucose during induced hyperphosphatasemia in all 3 dogs. Variations of such magnitude never occurred in the control experiments previously mentioned.

C. *The Influence of Hyperphosphatasemia on the Intravenous Glucose Tolerance Test.* 1. A few days after performing an intravenous glucose tolerance test, the test was repeated on the same animal following the intravenous injection of phosphatase. The results are shown in Fig. 2B. In 2 of 3 dogs, definitely prolonged and elevated curves followed the phosphatase injection. In the third animal (Subject A) the results were similar, though less striking.

2. In order to eliminate the possibility of a non-specific protein effect of the phosphatase injections, a phosphatase solution was inactivated by heat for 1 hour at 50° C. and the experiment carried out as described above. The curves obtained were almost identical (Fig. 2 C.).

3. The experiments using the intravenous glucose tolerance test were repeated with phosphatase solution III which had been prepared from cattle intestines. The results, shown in Fig. 2D, illustrate the failure of induced hyperphosphatasemia with this preparation to exert any influence upon the tolerance to glucose.

D. *The Effect of Hyperphosphatasemia Upon the Peripheral Disappearance of Glucose.* In order to study the influence of the injected phosphatase on the peripheral uptake of glucose, 2 dogs were eviscerated and the blood sugar maintained by the continuous injection of glucose delivered by a constant injection apparatus at a rate of 0.125 gm. of glucose per kilo per hour. Following a control period, 10 cc. of phosphatase solution were injected intravenously. The results are shown in Fig. 3, and illustrate the failure of phosphatase significantly to affect the rate of peripheral utilization of glucose even at very high serum alkaline phosphatase levels.

E. *The Effect of Induced Hyperphosphatasemia on Fructose Metabolism.* Blood glucose and fructose levels were determined during oral and intravenous fructose tolerance tests before and after the intravenous administration of phosphatase. The protocols are shown in Table 4. Phosphatase caused a more rapid conversion of orally administered fructose to glucose. When the fructose was administered intravenously, the curves before and after phosphatase showed no significant differences.

Discussion. The data presented fail to demonstrate any significant alteration in carbohydrate metabolism in patients with osteitis deformans as measured by their response to the oral and intravenous glucose tolerance tests. Though our results appear to be at variance with the work of Moehlig and his associates[11], who found a "diabetic dextrose tolerance curve" in 88% of their patients with Paget's disease, the difference is one of interpretation of data rather than an absolute dis-

crepancy. When the composite curve for the oral glucose tolerance of 31 of their patients (11 D) was superimposed on our oral glucose tolerance graph, their similarity becomes apparent (see Fig. 1A). The average age for their group of patients was 53.8 years and for our group 59.7 years. The importance of comparing glucose tolerance tests done in any investigation of patients of middle or old age with a control group of similar age must be emphasized. Several workers[6] have demonstrated a gradual depression of oral glucose tolerance with progressing age. Our control group of patients, whose ages ranged from 58 to 84 years, showed a slight but significant depression of tolerance to intravenously administered glucose. This finding of diminished intravenous glucose tolerance in older age groups has recently been confirmed by Smith and Shock[15]. A relationship between the degree of prolongation of the glucose tolerance test and the age of the control subjects or the patients with Paget's disease could not be demonstrated. There was no statistically significant difference between intravenous glucose tolerance curves in the control series and in the Paget's group. Had we, however, compared the results in our patients with the criteria of normal glucose tolerance tests established, in the main, on young adults, we would have concluded that Paget's disease caused an aberration in carbohydrate metabolism.

The studies of glucose tolerance in dogs with induced hyperphosphatasemia suggest that transient alterations in carbohydrate metabolism can be produced by this experimental technique. Both the oral and intravenous tests showed elevated prolonged blood sugar curves when the serum phosphatase was maintained at an increased level of enzyme activity by the intravenous injection of potent phosphatase solutions. The injected phosphatase may have altered glucose tolerance by increasing the rate of dephosphorylation of hexose phosphates, or it may have exerted a less specific glycogenolytic action.

The failure of phosphatase solution III, prepared from cattle intestine, to act as solution I and II, prepared from dog intestine, suggests a species-specific activity of phosphatase. Such an assumption would be warranted, however, only if this finding proved to be consistent in a larger series of experiments. The experiments with fructose were designed to test the hypothesis that elevated phosphatase hastens dephosphorylation. Since fructose must be converted into the glucose phosphates in its metamorphosis to glycogen, one might expect it to be rapidly converted to glucose in the presence of increased phosphatase. This occurred after the oral administration of fructose but not after its intravenous administration. The disappearance rate of blood glucose in eviscerated dogs was not altered by high phosphatase blood levels, showing that the mechanism of action was not a peripheral block to the entrance of glucose in body cells.

The experimental alteration of glucose tolerance in dogs with hyperphosphatasemia and the normal glucose tolerance in patients with osteitis deformans and hyperphosphatasemia are not necessarily contradictory findings. Experimental osteitis deformans was not produced in the experimental animals, and their abnormal glucose tolerance curves may well represent a non-specific glucogenic mechanism rather than a specific enzymatic acceleration of dephosphorylation.

The concept of Paget's disease as basically endocrinopathic in origin has been based chiefly on the reported

abnormal glucose tolerance, a finding that we have failed to confirm.

Summary. 1. Oral and intravenous glucose tolerance tests performed on patients with osteitis deformans (Paget's disease of bone) did not differ significantly from similar tests performed on normal control subjects of similar age groups.

2. Increasing age serves to depress tolerance to both orally and intravenously administered glucose. Studies, therefore, utilizing glucose tolerance tests in middle or old-age patients must be compared with standards derived from subjects of similar ages, rather than from young adults.

3. A method of experimentally elevating serum alkaline phosphatase in dogs by the intravenous injection of phosphatase solutions is described.

4. Experimental hyperphosphatasemia altered glucose tolerance in dogs when performed by either the oral or intravenous route, though the results were not consistent when phosphatase solutions prepared from cattle rather than dog intestines were used.

5. This study does not support an endocrinal concept of the origin of Paget's disease.

ADDENDUM. Since preparing this report the author found reference to work, as yet unpublished, by Professor William Kerppola of Finland (Joslin, E. P., *et al.*, New England J. Med., **238**, 438, 1949). This investigator prepared a highly active phosphatase solution and induced hyperglycemia and depressed glucose tolerance by injections into rabbits. His publication is awaited with great interest.

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THE USE OF ORAL MERCUHYDRIN COMBINED WITH ASCORBIC ACID IN CARDIAC DECOMPENSATION*

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Certain observations have been made by several investigators on the use of various oral mercurial diuretics; they may be effective in the management of mild cardiac failure, may prolong the interval between injections of a mercurial, and may provide a treatment which eliminates repeated severe diuresis and rapid elimination of chlorides[1,2,5,6,9]. This paper is a report of our experience with a new oral mercurial diuretic.

The mechanism of diuresis by inorganic mercurial compounds is thought to be a prevention of reabsorption of the sodium ion by the renal tubules. Animal experiments by Handley to determine the absorption and excretion of orally administered Mercuhydrin have ascertained that the total amount of mercury recovered in the urine and feces is approximately two-thirds of the mercury administered by mouth[7]. Mercury is apparently eliminated in the urine and feces almost completely within 48 hours after the first dose is administered. Most of the remainder is eliminated as a volatile organometallic compound in the expired air.

The preparation of a tablet combining an inorganic mercurial compound (Mercuhydrin) and ascorbic acid** was conceived mainly on the basis of studies done by one of us (C.F.S.) on the diuretic effect of vitamin C administered orally in both normal persons and those with edema[8]. Ascorbic acid, given orally, is almost completely absorbed, and the rate of excretion varies inversely with the extent of tissue concentration.

An investigation of the diuretic effect of ascorbic acid was made in 10 hospital patients well controlled with bed rest, a standard diet with a measured vitamin C content and a relatively stable fluid balance for 3 days prior to giving the ascorbic acid. No other diuretic was used during the 6-day period of study. Liquids were regulated at 1600 cc. in 24 hours and the urine output was recorded. All were given a daily supplement of 500 mg. of ascorbic acid by mouth in divided quantities. It had previously been demonstrated that, regardless of vitamin C reserve, a person can be saturated by a peroral dose of 3 gm. of ascorbic acid in 6 days[10]. The diuretic action corresponded to the time of saturation, usually on the 3rd or 4th day if there was a normal tissue reserve. All patients had a diuresis as determined by comparing the 3-day average output at the height of response (usually the 3rd, 4th and 5th days) to the same period average

* This research was aided by a grant from Lakeside Laboratories Inc.

** Tablet Mercuhydrin with ascorbic acid (Lakeside Laboratories Inc.).

before giving vitamin C. The actual increase in output was 250 cc. to 1000 cc. in 72 hours.

Other observations instigated the preparation of this tablet: The establishment of the detoxifying effect of vitamin C on heavy metals as determined by other investigators[3], our investigation in animals of the detoxifying effect of ascorbic acid on the intravenous mercurial preparations[4], the discovery that the combination of an inorganic mercurial compound and ascorbic acid intravenously may result in an enhanced diuresis up to 50% or more as compared to the mercurial preparation alone[4,8], and the fact that vitamin C is non-toxic regardless of the dose or route of administration.

This study comprises the results obtained in 50 patients, from the Cardiac Clinic of Hermann Hospital, Baylor University College of Medicine, and private practice. The patients studied represented congestive heart failure due, in the majority, to common causes, namely valvular, hypertensive and arteriosclerotic heart disease. Their ages ranged from 12 to 60. The number of tablets administered ranged from 1 to 3 a day, each tablet containing 60 mg. of Mercuhydrin (equivalent to 19.5 mg. of mercury) and 100 mg. of ascorbic acid. A restricted sodium diet and digitalis were given, when indicated, to all patients. It was noted that the response to tablets was more satisfactory in the private patient group, apparently due to their cooperation in adhering to the program of treatment.

Case Reports (Selected from the Private Patient Group). CASE 1. HYPERTENSIVE CARDIOVASCULAR DISEASE WITH ANGINA PECTORIS, LEFT BUNDLE BRANCH BLOCK AND PERSISTENT LEFT SIDED FAILURE. Mrs. W. C., 72, was seen in November, 1947, with severe left ventricular failure. She had had arterial hypertension for at least 15 years. Eighteen months previously she had experienced protracted chest pain considered to be myocardial infarction. Since then she had been practically inactive due to angina pectoris on the slightest effort. Her medication consisted of 0.1 mg. of Digitoxin daily. The patient was moderately obese and presented evidence of advanced arteriosclerosis. The signs of severe left ventricular failure were present and the liver was palpable and tender but there was no peripheral edema. The arterial systolic pressure was 180 and the diastolic 120 mm. of mercury. The cardiac apex was 2 cm. lateral to the mid-clavicular line. An electrocardiogram revealed a left bundle branch block.

She was placed on a restricted sodium diet and given 1 cc. Mercuhydrin with 500 mg. of ascorbic acid intravenously. Several hours after the injection she became nauseated, mentally confused, and developed pain in the lumbar region and lower extremities. There was no appreciable alteration in objective evidence of congestive failure. The state of unusual ill being gradually subsided, and 1 week later 1 cc. Mercuhydrin was given intramuscularly with less disturbance. Xanthine preparations and ammonium chloride were ineffective and left-sided failure persisted in spite of restricted sodium and Digitoxin until Januaury, 1948, when 1 tablet of Mercuhydrin with ascorbic acid was given daily and tolerated without ill effect. There was a loss of 12 pounds in weight with disappearance of the pulmonary congestion and a substantial decrease in the blood pressure during a period of 3 weeks. The patient regained her sense of well being and is able to be moderately active without angina or dyspnea. The Mercuhydrin with ascorbic acid was continued except for short periods when the tablet was not available. On these occasions the patient sensed an increase in respiratory disturbance by the 2nd or 3rd day and measurable evidence of left heart failure became evident within one week.

CASE 2. CALCIFIC DISEASE OF THE AORTIC AND MITRAL VALVES WITH CONGESTIVE HEART FAILURE. Mr. T. B., 61, was seen in August, 1948, with severe congestive heart failure. He had been healthy, without evidence of earlier rheumatic disease, until 1945, when he became breathless on exertion. This disturbance gradually increased in severity and nocturnal coughing, dyspnea, orthopnea and dependent edema developed. The patient was well proportioned and presented evidence of gross congestive heart failure, with hepatic and pulmonary engorgement and peripheral edema. The cardiac apex was 3 cm. lateral to the mid-clavicular line in the 6th inter-

costal space. Grade III to IV systolic murmurs were present over the mitral and aortic areas. The blood pressure was 128/110. The vital capacity was reduced to 1500 cc. Calcification of the aorta was noted on fluoroscopic examination. An electrocardiogram indicated a predominating sinus mechanism with frequent multifocal premature ventricular systoles, abnormal left axis deviation and left ventricular hypertrophy.

On a regimen including a restricted sodium diet, Digitoxin and Mercuhydrin with ascorbic acid intravenously at indicated intervals, a rapid and satisfactory response was obtained. There was a loss of 25 pounds in weight, a decrease in blood pressure to 110/70, and a rise in vital capacity to 3100 cc. during a 4-week period. One tablet of Mercuhydrin with ascorbic acid daily, with moderate sodium restriction, Digitoxin, and an occasional injection of Mercuhydrin has allowed the patient to continue his duties through the past 6 months without congestive failure.

Case 3. Coronary Heart Disease with Left Ventricular Failure. Mr. G. S., 57, was seen in October, 1947, with moderate hypertension, angina pectoris and severe left ventricular failure. Typical angina of effort had recurred occasionally during the previous 6 years, but for the past 3 months these episodes had become increased in frequency and severity. He also had become more and more dyspneic on exertion. The patient was of average build and had a regular rhythm at a rate of 60 per minute with a blood pressure of 190/100. Numerous moist rales were present at the lung bases. The heart appeared normal on physical, electrocardiographic and roentgenographic examination.

A restricted sodium diet, Aminophyllin and Mercuhydrin with 500 mg. ascorbic acid intravenously resulted in a loss of 10 pounds in weight with disappearance of signs of left sided failure and a reduction in blood pressure to 130/80. The anginal episodes ceased and, after the 4th injection, these were discontinued because of disagreeable cerebral and gastrointestinal symptoms. Approximately 4 weeks later, the patient experienced a recurrence of angina which rapidly developed into uncontrollable angina decubitus. Left sided failure also recurred and became persistent. Through this period and later, electrocardiograms revealed myocardial ischemia. A Mercuhydrin with ascorbic acid tablet was then administered daily with improvement of congestive failure and a weight loss of 18 pounds. The angina gradually subsided with only an occasional mild episode as the patient became stabilized on this regimen. There have been no signs of ventricular insufficiency for 6 months, and the electrocardiogram has returned to normal.

Case 4. Recurrent Pulmonary Embolism and Myocardial Infarction with Left Ventricular Failure. Mr. E. M., 70, was seen in June, 1948, with acute pulmonary embolism. This was the 4th such episode during the preceding 6-month period, each sudden in onset with severe chest pain, dyspnea and hemoptysis. The patient was of a slight build, with evidence of extensive peripheral arteriosclerosis. There was dullness to percussion over the right scapular region and bilateral rales. A teleorocentgenogram revealed, in addition to pulmonary congestion, an area of consolidation at the right base. The electrocardiogram indicated an old infarction on the lateral surface of the left ventricle.

The patient responded to anticoagulant therapy, a restricted sodium diet, and intramuscular injections of Mercuhydrin as indicated, but convalescence was protracted. Left ventricular failure persisted and he was then given 1 tablet daily. This has been continued and he has remained moderately active without evidence of left ventricular failure.

Case 5. Rheumatic Heart Disease with Chronic Congestive Failure. Mr. C. B., 54, was seen in September, 1948, in gross congestive heart failure. He had recurrent episodes of rheumatic fever between the ages of 12 and 32 years. Dyspnea on exertion had been present throughout adult life and congestive failure occurred one year previously. He had a variable course from that time on a diet restricted in sodium, digitalis and various diuretics, but apparently had never been rid of congestive failure.

The patient presented the picture of chronic congestive heart failure with peripheral edema, pulmonary edema, hepatomegaly and ascites. Cardiac enlargement apparently involved all chambers. Murmurs indicating mitral stenosis and insufficiency and aortic stenosis were present and disease of the tricuspid valve was suspected. An electrocardiogram revealed auricular fibrillation with an idioventricular rhythm at a rate of 60 per minute.

Bed rest, restricted sodium, ammonium chloride tablets and daily intravenous injections of 2 cc. Mercuhydrin with 500 mg. ascorbic acid produced no satisfactory diuretic response. Intramuscular injections of Mercuhydrin were slightly more effective. Then Mercuhydrin with ascorbic acid tablets were given daily in addition to 1 cc. by vein, and dependable diuresis was established with a rapid loss of 16 pounds in weight. The

tablet has been well tolerated and has been continued, being augmented by intravenous injections at 3 to 5-day intervals. The patient has been relatively free of congestive failure on moderate physical activity but with persistent evidence of hepatic cirrhosis.

Case 6. Chronic Pyelonephritis, Hypertension and Coronary Heart Disease with Congestive Failure. Mr. O. P., 62, was seen in March, 1949, in severe congestive heart failure. He had been well until August, 1948, when there occurred a severe, acute urinary tract infection which responded slowly to treatment. On resuming activity he noted dyspnea on exertion which became increasingly severe until January, 1949, when orthopnea and generalized edema developed. Satisfactory response to hospitalization and treatment for congestive heart failure at that time proved to be only temporary. The patient was a large man, moderately obese, dyspneic at rest, with unhealthy pallor. Extensive peripheral and pulmonary edema were present and the liver extended 6 cm. below the costal margin. The blood pressure was 160/110. The cardiac apex was 3 cm. lateral to the mid-clavicular line and orthodioscopy indicated the enlargement to involve mainly the left ventricle and left auricle. An electrocardiogram revealed prolonged intraventricular conduction and generalized non-specific T wave changes. Blood examinations revealed moderate hypochromic anemia with normal nitrogen and serum protein levels. The urine contained a moderate amount of albumin, blood cellular elements and granular casts.

Congestive failure was considered of paramount importance and the patient was placed on a restricted sodium, low calory, high vitamin diet regimen and Mercuhydrin by intramuscular injection was tried for effect. Satisfactory diuresis occurred with a prompt loss of weight and fall in blood pressure. Orthopnea and dyspnea disappeared as the congestive failure subsided. One tablet of Mercuhydrin with ascorbic acid was then begun daily. This has been well tolerated and has been continued with the patient remaining free of congestive failure on moderate physical activity.

Results. Certain general observations were made:

1. The tablets of Mercuhydrin in combination with ascorbic acid were equally as effective as a tablet containing 120 mg. of Mercuhydrin alone (equivalent to 39 mg. of mercury) and there were significantly less side reactions when the combination was used.

2. A relatively large total number of tablets of Mercuhydrin, combined with ascorbic acid may be given without toxic effect, that is, daily use of as many as 3 tablets for as long as 18 months (recommended optimal daily therapeutic dose is 1 to 2 tablets).

3. The tablets were frequently satisfactory in controlling mild or moderate congestive failure after maximum compensation had been obtained by injectable Mercuhydrin. They were often unsatisfactory in controlling severe cardiac decompensation.

4. The tablets were frequently satisfactory in correcting mild congestive failure when used as initial therapy. They were unsatisfactory for correcting moderate or severe failure when used in this manner.

5. Toxicity, which occurred in approximately 25% of our series, was mild and transient in the large majority of instances. It consisted of salivation, nausea, with occasional vomiting, abdominal tenesmus, increased frequency of defecation (from 2 to 6 movements daily), and bloody diarrhea in 3 patients. Toxicity usually disappeared promptly on discontinuing the tablets.

6. There were 15 failures in control of congestive failure; and of these, 10 were moderately well controlled by injectable Mercuhydrin. Three patients died, 2 of cardiac decompensation and 1 of uremia. Two patients did not remain in contact with the clinic.

7. There was no apparent deleterious effect in persons with renal insufficiency as a result of chronic glomerular nephritis and the blood non-protein nitrogen content was unaffected as related to the administration of the tablets.

Investigators in other clinics have studied the effect of these tablets in more than 300 patients and have reported satisfactory results in approxi-

mately two-thirds of those treated for cardiac decompensation. The results were unsatisfactory or uncertain in the remaining third. They have also reported that the plain Mercuhydrin tablets were not so satisfactory as the combination of Mercuhydrin and ascorbic acid, both in regard to therapeutic effectiveness and toxic manifestations.

Summary. These studies indicate that Mercuhydrin with ascorbic acid tablet is a reasonably safe therapeutic agent and may either be used alone or as a supplement to parenteral therapy to assist in the control of congestive heart failure.

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PROGRESS
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GYNECOLOGY AND OBSTETRICS

UNDER THE CHARGE OF

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NATURAL CHILDBIRTH

A Summary of Results and a Commentary on Recent Literature

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THE term "Natural Childbirth" remains somewhat unfortunate in that it suggests that the process of childbirth can be readily defined as natural or unnatural, an obvious impossibility. The term also has implications that easy and relatively painless childbirth occurs in primitive tribes and peoples in a less cultured or "natural" state, which is by no means substantiated. Unfortunately this latter meaning has been seized upon by certain writers for the lay press and woven into a fabric which has been offered to the public under the blanket of "Painless Childbirth". The truth is that none of the proponents of the Natural Childbirth program has ever claimed that childbirth should be conducted without the availability of anesthetic aids or that the regimen renders labor devoid of pain.

The Natural Childbirth program as it has been developed at the Grace-New Haven Community Hospital (University Service) is essentially a training program for expectant mothers. It emphasizes normal childbirth's being a normal physiological process accomplished by muscular effort, that education of the woman for the act of childbirth and the responsibilities of motherhood is important, and that sympathetic and skillful attention during active labor should be continuously maintained. As the program has developed in our hands a definite portion of it has been directed toward the expectant father so that the emotional aspects and responsibilities of parent-

hood of both husband and wife may be better understood and family life begun more auspiciously.

The program at New Haven, which will be given in outline forthwith, is based primarily on the following assumptions:

1. Emotional influences play a major role in successful and satisfactory pregnancy and labor.

2. A program of mental and physical training for expectant mothers is useful in favoring a normal process of labor and in reducing the discomfort which accompanies that event.

3. The great majority of women who have been educated in this way and have adequate "support" during labor will go through that experience in a more satisfactory way and with a sense of accomplishment which is important to them.

4. The program has further implications in that, by means of classes held for both wives and husbands, a contribution is being made toward the satisfactory establishment of family life.

The essential details of the program are as follows:

The registration, or initial visit, of the expectant mother follows a conventional pattern of review of patient's medical history and the physical examination. In addition, an attempt is made to assess her personality, to reassure her as to her physical condition and to make her feel that the clinic staff of nurses and doctors are taking a real interest in her. She is encouraged to ask questions. On the following visit, in addition to the regular antenatal examination, she attends, as a member of a small group, a short lecture given by a doctor on the anatomy and physiology of pregnancy. Group discussion is promoted in all class work.

During the next regular visits the patient is taught, by a nurse, exercises which are designed to increase the elasticity and tonus of the back, abdominal and perineal muscles, and also breathing and position exercises which aid in relaxation.

A member of the pediatric staff interviews each woman during her prenatal period to become better acquainted and to give her a better understanding of various aspects of child care. A dietitian sees each patient at least once and a medical social worker is available.

At the 8th calendar month of pregnancy the mother attends a second class given by a doctor, at which labor is explained and the effects of tension and the relationship of anxiety and pain are discussed. The group visits the maternity division at this time, sees the delivery room and is informed about the hospital admitting routine. The exercise classes held at this period are also devoted to labor techniques. Mistakes in exercise practices are corrected and labor is rehearsed.

During the last trimester 4 evening classes are held, 1 for husbands and 3 for husbands and wives together. The first of these, given by an obstetrician and attended by husbands, is devoted to a discussion of pregnancy. The subsequent 3 classes are given by an obstetrician, a pediatrician, and a psychiatrist, respectively, and are attended by both husbands and wives. In the first of these the labor process is again explained and in the remaining 2 attention is directed toward problems of the newborn, parent and child relationships, and the start of family life. Group discussion is promoted and is always an important feature of these classes.

The most important part of the program we have referred to as *support during labor*. During active labor the patient is never left alone; attendants

help instruct the mother to carry out the relaxation and other techniques that she has learned in classwork. The husband is allowed in the labor room, which is a single unit, and may remain until his wife is taken to the delivery room. Following delivery, if the mother has elected rooming-in accommodations, wife, husband, and baby usually go to that unit together.

In the 3 years in which the plan has been in operation in most of its essential details, we have cared for approximately 1500 mothers through full-term pregnancy. A recent analysis shows that, of 1100 women who were delivered vaginally on this service, 90 (8.2%) required operative intervention. Of the 1010 who delivered spontaneously, 500 (48.5%) received some medication in the first stage of labor, usually a single dose of an analgesic (demerol). During the second stage 552 (51.7%) were given some inhalation anesthesia, usually whiffs of nitrous oxide and oxygen. Many of these patients received such assistance during both stages so that only approximately 25% went through labor without any medication whatsoever. The drugs so administered are usually small in amount and, as a result, of the 1010 women who delivered spontaneously 948 (93.9%) were fully conscious at the time of delivery.

A further consideration of results gives the distinct impression that labor is shorter, there is less abnormal bleeding and less postpartum fatigue in these patients. The necessity for resuscitation of the newborn is practically non-existent.

The reaction of the mothers (and the fathers) to the program has been favorable, usually enthusiastic. We have attempted to evaluate some of the reactions on the part of the mothers by a simple questionnaire which is filled out on the 3rd or 4th postpartum day, and which will form the basis for further study. The reaction of the attendants, upper staff, house staff, nurses, student nurses and medical students has also been interesting. There is general agreement that attendance on these patients is more interesting and of higher educational value than obtained previous to the inauguration of the program.

Though a summary of the advantages of the program has been set down elsewhere[7], its repetition may be useful.

1. An educational program incorporating physical training for prospective mothers has important advantages during pregnancy and labor.

2. Not only does a better understanding by the patient of the physiologic process of pregnancy and labor, which may be gained by instruction from physicians and nurses, reduce anxiety by increasing the confidence of the patient, but the use of physical therapy techniques definitely aids in the muscular effort of labor.

3. We do not think that the program we have established is fundamentally new; we view it rather as an attempt to emphasize and bring into practical use certain aspects of present day knowledge of the psychology and physiology of pregnancy and childbirth.

4. Objections have been raised against such a program, to the effect that it is impractical because of the time that physicians and nurses must devote to it. We do not consider this criticism valid, because the use of the class method of instruction actually takes but little increased time. As far as attention in labor is concerned, it is our opinion that patients in active labor should not be left alone with any regimen.

5. We recognize that fear and anxiety play a large part in the cre-

ation of tension, which has undesirable effects, but we do not place undue emphasis on this aspect. We prefer to think of our program in a broad and more positive sense as being directed toward childbirth with understanding and support rather than toward childbirth without fear.

In a survey of recent literature which has a bearing on this subject the writings of Grantly Dick Read occupy the foremost place. An excellent summation of the many contributions of this author is to be found in his monograph, The Birth of a Child[4] (1947). In this the author explains the general principles of teaching of physiological childbirth, the education of mothers, physical preparation and the conduct of labor. Emphasis is upon the teaching's being applicable only to normal labor, and the premises are based on clinical observation and deduction rather than theory. The author makes the following statement in the Introduction:

"The reactions of a woman to the changes that occur during pregnancy and labour have been considered more important than the reactions of a woman to the criteria of experimental science. I have resigned from controversy with those who have never given this teaching a fair and honest trial. It has brought happiness and safe motherhood to great numbers of women all over the world. There is adequate evidence that both pain and fear of childbirth have been reduced wherever this approach to motherhood has been employed. I feel justified, therefore, in turning a deaf ear to iconoclastic theorists and critics when results have proved beyond doubt the great service of these tenets to the women of our time."

Other important contributions to this subject are found in the writings of the late Helen Heardman. Her book, A Way to Natural Childbirth[1] (1948), gives in detail the exercise and relaxation techniques which as a physiotherapist she has found can be usefully applied to clinical obstetrics. To her observations this author brings an extended clinical experience both in the antenatal clinic and the labor and delivery rooms. Mrs. Heardman states that for childbirth to be natural, 3 factors are essential: 1, proper health (including sex) education; 2, proper preparation during pregnancy; 3, proper conduct of labor. Anesthesia or analgesia must be available and offered, though never forced. The choice lies with the mother herself.

An important pioneer in this country is Blackwell Sawyer, and his paper, Experiences with the Labor Procedures of Grantly Dick Read[5] (1946), will remain a landmark. Dr. Sawyer is a general practitioner with a deep interest in obstetrics. In summary he writes, "It would seem that the method of Grantly Dick Read, when carefully applied, is valuable in relieving the pain of normal childbirth in about nine out of ten women. The pain is relieved to a greater or less degree. Some women had no pain to speak of, to use their language, in the first stage, while others could not relax until Demerol was administered, after which the method succeeded. The effects during the second stage were very apparent, but the end of this stage required other help in some cases, that is, either ether anesthetic or novocain locally or both."

An important contribution to the psychological aspect of this subject is found in Menninger's paper, The Emotional Factors of Pregnancy[3] (1943). This author lists some of the fears that emotionally healthy women may have toward pregnancy, such as: 1, dread of becoming pregnant or a fear that perhaps she cannot become

pregnant; 2, fear founded on the timing of pregnancy (too soon after marriage, too soon after last child); 3, fear based on financial insecurity (the woman may be employed, may fear for her job); 4, fear of husband's antagonism or hostility to the child; 5, fear the pregnancy will ruin her health or her figure; and, 6, fear of the responsibility of taking care of the child. In addition are certain fears directly associated with pregnancy: fear of labor and anticipated pain; fear of death is not uncommon. He concludes, "The universality of pregnancy and its importance as one of the major experiences in the life of a woman, both psychologically and physiologically, justify an extensive study from the psychosomatic point of view."

Under the title, Psychology and Childbirth[2], Ernest Jones says a neurotic attitude can "make the patient hard to guide, control and help, and may have indirect effects on the course of labour itself; but . . . if her attitude toward her unborn child is purely positive, the course of pregnancy and the labour is, apart from physical complications, smooth, and is accomplished with love and pride."

The contributions of Wolff and Goodell[9], and Wolff[8] are important in any consideration of natural childbirth. The former state that "attitude and suggestion may modify both the pain threshold and the manner of reaction to pain," and that "attitude engendered by situation and experience may be accompanied in alterations in the pain threshold and in the manner of reaction to pain." In another communications Wolff states, "Instances of dissociation of pain perception from the pattern of reaction to pain are seen in the indifference to injury sustained during the excitement of games or combat, the apathy or 'quietism' that accompanies tissue damage during religious or mystical practices; the indifference to tissue damage during sexual excitement; the indifference to pain often witnessed during parturition in women who are confident in their physicians and desirous of bearing a child."

The more recent views of the author of this review are to be found in a recent monograph, Training for Childbirth[6], in which he concludes: "If wider changes in obstetrical procedure are made, they will come through obstetricians who believe that the underlying principles of the natural childbirth program are useful and fundamentally sound. It is the author's belief that, whatever type of delivery is elected, this type of psychological and physical preparation for childbirth is important and that it will be of help to every woman who is granted the experience and opportunities of motherhood."

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PEDIATRICS

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FETAL HEMOGLOBIN IN THE HUMAN: A REVIEW

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A DISTINCT difference between the hemoglobin of the fetus and the adult has been demonstrated, not only for the human, but for other mammalian species including the goat[13,33], the cow[3], the sheep[3,25] and probably also the rabbit[6] and the chicken[13]. In the human being the change over from fetal to adult hemoglobin begins to take place soon before birth and is usually completed by the first 6 months of infancy[6].

The differences between the 2 hemoglobins can be discussed under the following headings: 1, denaturation by alkali; 2, physico-chemical measurements; 3, immunologic reactions; 4, crystalline structure; 5, amino acid composition; 6, electrophoretic mobility; and 7, possible clinical applications.

These differences are comparatively subtle when compared with their fundamental similarities. Nevertheless, there are a few indications that these differences may be of significance in certain conditions in the newborn and early infancy.

DENATURATION BY ALKALI. Recognition of the 2 forms of hemoglobin with respect to infant and adult dates from the studies of von Korber[27] in 1866, who found that adult human hemoglobin changed to alkaline hematin quickly, whereas the conversion of human placental blood was more sluggish. Wakulenko[39] in 1910, using the umbilical cord blood obtained from a newborn infant, also demonstrated this difference in speed of denaturation. The observations were expanded several years later by von Kruger[28], who measured spectroscopically the rates of disappearance of the oxyhemoglobin band after adding a weak alkali solution to fetal and adult blood. More recently the spectrophotometer and the photoelectric spectrophotometer have been employed to measure more accurately the speed of denaturation of the 2 hemoglobins to alkali[6,14].

The simplest method of showing the reactions of adult and fetal hemoglobin to action of alkali is by means of a hand spectroscope[4,5,38]. With this instrument one measures the times of disappearance of the 2 typical absorption bands of a dilute oxyhemoglobin solution. The results correlated closely with those obtained with spectro-

photometric or photoelectric methods. By the latter, the times of disappearance of alpha and beta bands (578 and 540, respectively) can be more exactly noted.

The hand spectroscope method may be carried out in the following manner (modified from Helpern[17]):

Five cc. of a 1% solution of hemoglobin is prepared in a test tube. The color of this solution is pinkish red. Examination with the hand spectroscope shows the 2 absorption bands of the oxyhemoglobin spectrum plainly visible between lines D and E. One cc. of a 1% sodium hydroxide solution is then added. This will denature the hemoglobin, changing its red color to the brown color of alkaline hematin. The time required for the 2 distinct bands of the oxyhemoglobin spectrum to disappear can be followed with the hand spectroscope. The denaturation time for the hemoglobin of individuals beyond 4 to 6 months of age is usually about a minute, though maximum normal may be taken as up to 5 minutes. The denaturation time for the hemoglobin of the fetus or newborn infant, on the other hand, ranges from 1 to 3 hours. The contrasting time intervals for denaturation can be modified by using a more concentrated solution of blood and a weaker one of alkali.

Physico-Chemical Measurements. Fetal blood differs somewhat from that of the adult in both its oxygen and carbon-dioxide relationships. At gas tensions between 25 and 60 mm. of Hg., fetal blood absorbs oxygen more effectively than does mother's blood. At all gas tensions, fetal blood releases carbon dioxide more readily than does mother's blood[12,31]. These are helpful differences when viewed from the standpoint of placental interchange. Eastman and his co-workers[12] suggest that the differences can be explained by the laws which govern the diffusion of these gases through a wet membrane, but the differences in hemoglobin structure may also play a part.

Darling, Smith and associates[9] in 1941 confirmed the increased affinity of fetal hemoglobin for oxygen. These authors studied the dissociation curves and measured the electrolytes of the blood simultaneously, calculating the shifts in the oxygen dissociation curve from the changes in pH. Despite the presence of a more acid physiologic state in the fetus which would oppose oxygen uptake by the hemoglobin, there existed an inherent affinity for oxygen by the hemoglobin of the fetus. The latter force is somewhat greater so that fetal blood operates at an advantage in prompt saturation with oxygen in its passage through the placenta.

These conclusions were based on the study of 2 premature and 8 full term infants. It was noted that the differences in the salt concentration of the serum probably were too insignificant to explain the shift in the fetal oxygen dissociation curve. Thus the authors conclude that "if the cell membrane of the fetal red cell has the same characteristics of permeability as the adult cell, then the fetal hemoglobin has different properties".

Using the surface-pressure curves in dilute buffer solutions at various hydrogen ion concentrations, Brinkman and Jonxis[6] were able to show that these 2 forms have different isoelectric points and rates of spread in monolayers.

Jongbloed[21] attempted to demonstrate the difference between maternal and fetal hemoglobin by direct examination of the spectra in ultraviolet light. No differences were found in 5 experiments.

In spectral-absorption studies Jope[23] found the prominent 5-structure band due to tryptophane to be at 289.8 millimicrons in human fetal hemoglobin,

and at 291.0 millimicrons in human adult hemoglobin.

Immunologic Reactions. Soon after the turn of the century, French workers[11,19,29] demonstrated serologic changes following the injection of hemoglobin into other animals. Precipitins specific for the hemoglobin injected were found. This was confirmed later by Heidelberger and Landsteiner[15] in 1923 who were also able to demonstrate the presence of complement-fixing antibodies and anaphylactic antibodies to the hemoglobin injected. Hektoen and Schulhof[16] and other investigators[20] found that the antigenicity of the hemoglobin resided in the protein fraction, globin.

The serological differentiation of fetal human from adult human hemoglobin was made in 1941 when Darrow and her co-workers[10] prepared rabbit antiserums specific for these 2 forms of hemoglobin. Antisera obtained from rabbits prepared previously with umbilical cord hemoglobin showed positive precipitation reactions with both adult and cord human hemoglobin. After absorption with adult hemoglobin further precipitation occurred with cord hemoglobin but not with the adult hemoglobin. After absorption with cord hemoglobin no further precipitation was obtained. Antisera prepared with adult hemoglobin showed no further precipitation with either adult or cord hemoglobin after absorption with either hemoglobin. The authors concluded that in the human species the hemoglobin obtained from the umbilical cord at birth contains a mixture of at least 2 different and distinct hemoglobins. Fetal hemoglobin is not demonstrable in the adult. There is apparently general agreement with Haurowitz that the prosthetic moiety of adult and fetal hemoglobin is identical and that the differences lie in the protein fraction.

Electrophoresis. The electrophoretic mobilities and ultracentrifugal sedimentation constants of these 2 varieties of human hemoglobin proved to be different in an investigation conducted by Andersch, Wilson and Menten[1], who tested specimens from infants and adults. Umbilical cord samples from 5, 9 and 90 day old infants contained both hemoglobins. From the data it seemed evident that fetal hemoglobin made up the largest portion, with adult hemoglobin being present in a small amount. Hoch[18], on repetition of the electrophoretic studies, noted a slight but less marked difference than reported by the above authors.

Crystalline Structure. In 1930, Perrier and Jannelli[35] described a difference in the crystalline structure of neonatal and adult hemoglobin. This had been confirmed more recently by Kendrew[26] and by Jope and O'Brien[24], with both sheep and human blood. The crystallographic differentions have been carried out both by Roentgen-ray diffraction studies and by direct crystallization of reduced hemoglobin and of methemoglobin. These make it evident that the 2 forms possess different molecular architecture. Adult HbCO, HbO_2 and MetHb form orthorhombic crystals, whereas fetal HbCO, HbO_2 and MetHb form triclinic (or monoclinic) crystals. The respective Hb crystals also differ strikingly from each other, in formatives still to be determined. The solubility curves of these crystals, when dissolved in salt solutions at various temperatures, were individually different from each other.

Amino-Acid Composition. Porter and Sanger[36] investigated chemically the free alpha amino groups of several sources of hemoglobin, using the technique of partition-paper chromatography. Human fetal hemoglobin proved to have 2.6 terminal valine residues in contrast to the adult

hemoglobin which had a fixed number of 5 valine residues. There were about 47 free lysine E amino groups in fetal human hemoglobin and only about 43 such groups in the adult hemoglobin. It is believed that the number of free alpha amino groups gives the number of open peptide chains present in the protein molecule. There is, of course, the possibility of other molecular configurations, such as cyclic chains or chains branching off the carboxyl groups of glutamic or aspartic acid. The number of open peptide chains would be equal to the total number of chains in the molecule. It is likely that the open chains are determinants of immunologic specificity. The authors, however, could not show that the free alpha amino groups were the sole determinants of immunologic specificity. The total gross molecular structure in addition to its amino-acid composition determines the specificity of a protein. The molecular weights of both human fetal and human adult hemoglobin appeared to be identical, approximately 66,000.

Related differences between fetal and adult cow hemoglobins have been noted by Wyman[41]. The fetal form has more isoleucine and less methionine and histidine than the adult form. In strong phosphate buffers the fetal hemoglobin is 6 times more soluble than is the adult hemoglobin. There are, however, no demonstrable spectroscopic differences between fetal and adult cow hemoglobins.

van der Linden[32] seems to have similarly demonstrated differences in amino-acid composition with the use of a microbiologic method based upon the need of lactic acid bacilli for certain essential amino acids.

Schenck[37] in 1930 showed that there was a constant difference in the amount of certain amino acids in the globin of various species. In the human, the blood was found to have a higher arginine content in the globin fraction of hemoglobin in the newborn than in the adult. There was a rapid decrease in this amino acid with advancing age. This "age-curve" of the arginine content of hemoglobin may hold true as a general phenomenon of all body proteins in the newborn[7].

Schenck also found that these chemical differences between human globins corresponded to their different resistances to pepsin and hydrochloric acid.

Possible Clinical Applications. More recent evidence substantiates by indirect observation that this distinct molecular structure of hemoglobin in the immediate neonatal life is responsible for failure of expected hematologic responses. Watson, Stahman and Bilello[40] made a study of the sickling phenomenon in 452 consecutive negro newborn and their mothers. Eight per cent of the mothers and 8.4 % of the infants showed sickling. Within the immediate newborn period to the age of 4 months, the sicklemia was difficult to demonstrate (0.5% to 29.5%), and the time needed for maximum sickling was longer in those newborns demonstrating this phenomenon. These authors believed that the lack of sickling in the newborn was dependent upon the presence of fetal hemoglobin. The transition to more rapid sickling coincided with the approximate life cycle of the red blood cell, which is approximately 120 days. It is suggested that the presence of fetal hemoglobin may have a protective influence in preventing sicklemia in fetal life, despite the low oxygen tension that prevails in utero.

In 1948, Jonxis[22] stated that in erythroblastosis fetalis due to Rh incompatibility the fetal hemoglobin is broken down and disappears rapidly because "only those (erythrocytes) which contain foetal haemoglobin are

haemolysed". Furthermore, the adult form of hemoglobin is said to be present in abnormally large amounts in the blood of such newborn infants—up to 4 gm. per 100 cc. of blood. Erythrocytes containing the adult form of hemoglobin are said to be completely, or nearly completely resistant to the hemolysis which takes place in erythroblastosis fetalis.

Baar[2] has challenged the above views, claiming that destruction of erythrocytes in erythroblastosis fetalis is based upon their sensitization to Rh antibodies and is unrelated to the chemical constitution of the globin component of their contained hemoglobin. His objection is based upon his own and Mollison's[34] observations that the destructive process attacks transfused cells as rapidly as the infant's own cells. Hemoglobin of the fetal type appears to be formed in the rapid regeneration which takes place in these cases.

Many reports have been recently published pertaining to nitrite poisoning in the newborn period. Nearly all the papers deal with methemoglobinemia resulting from the ingestion of well water containing large amounts of nitrates and nitrites. In a recent review of the subject, it was found that all of the patients were under 6 months of age. Despite the evidence of methemoglobinemia in all these infants, the quantities of nitrate ingested varied considerably (66 to 1107 mg. per liter). A few preliminary experiments by one of the authors[30] (H.L.) seem to indicate that nitrite forms methemoglobin more readily with the fetal form of hemoglobin.

Cornblath and Hartmann[8] suggest that the ease of formation of methemoglobinemia in the newborn period is related to the presence of large numbers of nitrite-forming bacteria in the upper gastrointestinal tract. This makes a greater amount of nitrite available for absorption, and for formation of methemoglobin by oxidizing the ferrous form of hemoglobin to the ferric form.

Post Natal Replacement. Haurowitz[14] estimated that the blood of the newborn contained approximately 80% resistant and 20% non-resistant hemoglobin. Trought[38] stated that fetal hemoglobin was usually absent from the blood of the newborn by four months. Bischoff[4] observed that it disappeared more rapidly from the blood of full term infants than prematures.

From their studies with the alkaline denaturation method, Brinkman and Jonxis[6] concluded that the fetal more alkali-resistant form of hemoglobin begins to disappear directly after birth. At the age of 3 months, about 23% of the hemoglobin is still alkali-resistant. By 7 months this form of hemoglobin has disappeared entirely. They noted also that a different type of "resistant" adult hemoglobin seems to make its appearance at 3 years of age, and to persist into adult life. Helpern[17] has shown that the speed of denaturation of fetal hemoglobin has medicolegal importance. He has found the hemoglobin of human fetuses and very young infants, because of its alkali resistance, will give a false positive Hoppe-Seyler alkali test for the detection of carbon monoxide in the blood. He suggests that other standard tests be used for the determination of carbon monoxide in such blood. However the alkali test may be utilized to differentiate decomposed and dried human fetal blood stains from that of adult blood. Very old blood stains give uncertain results.

Summary. The hemoglobin of the newborn is made up of 2 forms: a fetal hemoglobin (approximately 80%) and a more mature or adult hemoglobin (approximately 20%). This fetal hemoglobin disappears from the blood after 4 to 6 months of life. The difference between fetal and adult hemoglobin

probably resides in the globin fraction of the hemoglobin compound and accounts for the following distinguishing properties of fetal hemoglobin: A greater resistance to alkali denaturation; a quantitative and qualitative different amino-acid content; distinct serologic reactions and electrophoretic mobility; a different crystalline molecular structure; and a difference in its affinity for oxygen and carbon dioxide. The possible clinical applications are discussed.

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BOOK REVIEWS AND NOTICES

BONE AND JOINT RADIOLOGY. By EMERIK MARKOVITS, M.D., Steiner Cancer Clinic, Atlanta, Ga. Pp. 446; 616 ills. New York: Macmillan, 1949. Price, $20.00.

THE Author has provided a discussion of a wealth of material, which is presented in a clear and concise manner, though often the subjects are presented too briefly and are not brought up-to-date. For instance, the discussion of inflammatory diseases is just about what one would have expected to read in the time before this modern period of chemotherapy and antibiotics. One rarely sees osteomyelitis today as it is described in this book. The same criticism applies to consideration of the nutritional deficiencies. Recent observations show other categories of rickets than are considered in this book. The discussion of eosinophilic granuloma does not include some of the more recent concepts about that condition.

In spite of the above the student in radiology and the radiologist himself will receive much help from this volume.

Unfortunately, both negative and positive illustrations are used. The former are to be preferred. The pen and pencil drawings are excellent. Some of the illustrations have been retouched without notation in the accompanying legends. E. P.

BREAST DEFORMITIES AND THEIR REPAIR. By JACQUES W. MALINIAC, M.D., Clinical Prof. of Plastic Reparative Surgery, N. Y. Polyclin. Med. School. Pp. 193; 119 ills. New York: Grune & Stratton, 1950. Price, $10.00.

IN this complete monograph on breast deformities and their repair one finds a rich background of historical information, a purposeful study of the anatomy of the breast and a classification of the types of breast enlargement, with an accurate correlation between the clinical appearances and microscopic changes.

The indications for surgical treatment are fully covered; one-third of the volume is devoted to surgical technique. The Author analyses 159 cases operated upon by his own 2-stage method with transposition rather than free grafting of the nipple. A clear evaluation of existing techniques of mammaplasty serves to reduce the confusion concerning the choice of operation. The importance of blood supply is stressed, and this point is the basis of his own work in this field.

This book will be most valuable to those actively engaged in the practice of surgery of the breast; it should be read by all interested in plastic surgery. H. R.

THE PRINCIPLES AND PRACTICES OF REHABILITATION. By HENRY H. KESSLER, M.D., in Collaboration with 21 Authors. Pp. 448; 132 ills., 1 color plate. Phila.: Lea & Febiger, 1950. Price, $9.00.

THIS book, the work of 21 contributors, presents the various approaches to rehabilitation of the chronically disabled individual. Surgical, physical, psychological and administrative techniques are described and their application to a wide variety of disabilities is covered. The individual approach is stressed.

The book is divided into 2 sections: Principles and Practices. Specific disabilities are considered under each heading. Chapters are arranged so that readers interested in specific conditions may read selected chapters. Typography, indexing and illustrations are good. References are included.

The book should be of interest to all physicians, particularly those dealing with disability evaluation and chronic disease. R. S.

MEDICAL DISEASES OF THE KIDNEY. By J. F. A. MCMANUS, M.D., Assoc. Prof. of Pathology, Medical College of Alabama. Pp. 176; 101 ills. Phila.: Lea & Febiger, 1950. Price, $6.00.

THE beautiful photomicrographs of this clinico-pathological study constitute its most entertaining and valuable feature—in fact, one turns with difficulty from them to consideration of the text. Special stains have been frequently used to good advantage, especially Gomori's method for alkaline phosphatase, which throws more light on phosphatase distribution in various diseases, and the periodic acid Schiff's (PAS) reagent technique for emphasizing the basement membrane and various carbohydrates in the sections. Thus improved ways of differentiating varieties of glomerular injury are provided. Very high magnifications clearly bring out minute details—in several cases not more than a third of one glomerulus occupies a

5 inch square (strange to say, no magnifications are given). Regrettable, also, is the omission of the stain used in practically all of the legends after Fig. 25. Mallory's aniline blue, selective silver stains (reticulin), da Fano's impregnation (Golgi element), acid orcein (elastica), all help to bring out the finer details of kidney histology in health and disease.

The text could be criticized for not including more evidence in support of assertions made, even when they are contrary to those based on presumably more copious sources (e.g. Bell's material from the Mayo Foundation). Nowhere have I been able to find the number of cases on which the Author has formed this or that concept. However, a recognized authority who has read the book from cover to cover finds no fault with the Author's findings and deductions. Typographical errors are regrettably numerous; the details of Figures 66 to 69 are distinctly below average.

The book will be of chief interest to the pathologist and the pathologically-minded clinician; however, the illustrations, which speak for themselves, suffice to make it seem necessary for every student of renal disease, whether pathologist or clinician, to have this book at his elbow. E. K.

PHYSIOLOGY IN DISEASES OF THE HEART AND LUNGS. By M. D. ALTSCHULE, Ass't Prof. of Medicine, Harvard Med. School. Pp. 368. Cambridge, Mass.: Harvard Univ. Press, 1949. Price, $5.00.

THIS book, written in review form, presents clearly the physiologic concepts of cardiac and pulmonary disease, with the greatest emphasis placed on the subject of chronic cardiac decompensation. Conclusions drawn from the numerous papers reviewed are set forth clearly and concisely. An extensive up-to-date bibliography is provided so that the reader may locate the data upon which the conclusions are based.

Not only is an understanding of the physiologic basis of the symptoms of heart and lung diseases provided, but also one may obtain an idea of the various investigative methods employed in obtaining these facts. The relative merits of such procedures are briefly set forth.

Though the Author states that this book was written primarily for the 3d and 4th year medical student, to bridge the gap between the clinical and preclinical years, it is an extremely useful text for anyone desiring to further his understanding of pulmonary and cardiac disease. T. S., Jr.

PROCEEDINGS OF THE FIRST NATIONAL CANCER CONFERENCE. Pp. 310; illustrated. New York: Am. Cancer Soc., 47 Beaver St., and Nat'l Cancer Inst., U.S.P.H.S., 1949. Privately distributed.

VARIOUS attacks on the problem of cancer are presented by leading investigators with different concepts and methods of exploration. The individual papers deal with such subjects as differentiation of guinea pigs with inbreeding, mutations, enzyme stains, studies of plant growth, functional endocrinology, concepts of carcinogenesis, and the problem of cellular differentiation. Some of the papers have but slight connection with cancer research.

There is an informative panel discussion on tumors of different parts of the body, including recent developments in basic research, new methods of diagnosis and therapy. The problems of cancer education, diagnostic clinics and cancer grants are also considered. The book provides a clear picture of the wide range of activities related to the solution of the cancer problem. I. Z.

CLINICAL PATHOLOGY, APPLICATION AND INTERPRETATION. By BENJAMIN B. WELLS, M.D., Ph.D., Prof. of Medicine, Univ. of Arkansas. Pp. 397; 32 ills. Phila.: W. B. Saunders, 1950. Price, $6.00.

THIS book is designed to assist the clinician in the selection of laboratory procedures useful in the diagnosis and control of various diseases and in the interpretation of the laboratory results obtained. Techniques are deliberately omitted except for the procurement of material. Unfortunately, electrolytes of the serum are still expressed in the older manner of milligrams instead of the newer usage of milli-equivalents; reference is made to CO_2 capacity rather than content; several important recently introduced concepts have failed to obtain recognition. Some of these defects, however, are inescapable in a rapidly developing field. The book is undoubtedly a useful compendium of well established interpretations of laboratory findings and as such of service to the clinician.

J. A.

CLINICAL RADIATION THERAPY. Edited by ERNST A. POHLE, M.D., Prof. of Radiology, Univ. of Wisconsin. 2d ed. Pp. 902; 314 ills., 1 color plate. Phila.: Lea & Febiger, 1950. Price, $15.00.

ALMOST every radiologist in this country must be familiar with the first edition of this

book and will welcome this new one. The Editor has a number of excellent contributors and, throughout the book, has exerted the editor's prerogative when his opinion is at a variance with a contributor or when he wishes to emphasize an important point brought out in the discussions of the various conditions.

Almost every phase of roentgen and radium therapy is considered, but none of the Authors discuss the use of radioactive isotopes in treatment. References to them are made but no personal impressions are given. It is entirely possible that the contributors have not had any experience with radiophosphorus or radioiodine, radiosodium or radioactive cobalt. The two supplements prepared by Dr. Charles Martin on radium dosage are most helpful and help to round out the value of the new edition.

Dr. Pohle has done an excellent job and the publishers have shown their usual good workmanship. The print is very acceptable and the illustrations are good. The book will be valuable to anyone interested in radiology or cancer therapy. E. P.

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES. Vol. 50, Article 7, Pp. 679-814. ALLERGY. By ARTHUR F. COCA and 8 other Authors. Pp. 135; illustrated. New York: The Academy of Sciences, 1949. Price, $2.50.

TEN articles on various phases of the subject as discussed at a conference on allergy in April, 1947. The first 7 present current views and knowledge concerning anaphylaxis, classification of allergic diseases and their manifestations in animals, serum sickness, atopic allergy, allergy of infection, and immunologic changes brought about by fungi and their products. The last 3 deal with so-called familial non-reagenic food allergy (idioblapsis), a concept propounded by Coca that has received little acceptance, and its even less accepted and less acceptable treatment by unilateral sympathectomy (in turn connected with Coca's belief that hypertension is primarily allergic). R. K.

INTRODUCTION TO NEUROPATHOLOGY. By SAMUEL PENDLETON HICKS, M.D., and SHIELDS WARREN, M.D., Depts. of Pathology, Harvard Medical School and New England Deaconess Hospital. Pp. 494; illustrated. New York: McGraw-Hill, 1950. Price, $10.00.

THIS book represents the first attempt of American authors to approach neuropathology from the standpoint of the general pathologist. Functional and dynamic aspects are emphasized. The mechanisms of the circulatory and traumatic accidents as well as the metabolic disturbances underlying nervous diseases are aptly discussed. Excellent illustrations and useful tables accompany the text. The gross appearance of changes is more stressed than in the usual texts. Neurologists and neuropathologists may wish more histological details on the various forms of cortical and subcortical arteriosclerosis and their relations to clinical pictures, on the types of senile dementia, on paralysis agitans and chorea, on paresis and its place among the syphilitic diseases, its histology before and after fever treatment, the influence of antibiotics on the pathological picture in meningo-encephalitis and abscess, the neurological and neurosurgical implications of certain neuropathological conditions; but this book does not pretend to be more than an introduction to neuropathology. It is certainly an important right step in that direction. F. L.

AVIATION MEDICINE. By KENNETH G. BERGIN, M.D., British Overseas Airways Corp. Pp. 447; 131 ills. Balt.: Williams & Wilkins, 1949. Price, $7.00.

THE status of aviation medicine as an additional discipline in medical education is indicated by the publication of this British equivalent to H. G. Armstrong's textbook on aviation medicine. This volume is written specifically for the medical profession, presenting in quite readable and compact form the physiological, medical, psychological and epidemiological problems associated with aviation. The 35 chapters are well organized and the material is arranged to give quickly the information, conclusions and practical answers to questions, where the latter is possible. There are bibliographies at the end of every chapter, which compensate to some degree for the brevity and sketchiness of some parts in this comprehensive review. The abundant illustrative material includes many excellent photographs, some in color. In addition to the useful index there are 5 appendices on International Civil Aviation Organization medical requirements, table of average body build, epidemiological maps, international certificates of inoculation and vaccination and yellow fever inoculation centers (in British Isles). Dr. Bergin, in addition to his professional and technical qualifications, was medical officer in charge of operational aircrew personnel in the Royal Air Force. S. G.

A STORY OF NUTRITIONAL RESEARCH. By SIR EDWARD MELLANBY, K.B.E., F.R.S. Abraham Flexner Lectures Series No. 9. Pp. 454; 73 ills. Balt.: Williams & Wilkins, 1950. Price, $5.00.

IN 1915, the Author began studies on the etiology of rickets. This volume is a clear, and interesting exposition of his many contributions, through 3 decades, to knowledge of the role of vitamins A and D in bone growth. The early lectures trace the demonstration that nervous defects can develop through distortion of nervous tissue arising from hyperplastic bone growth. It is shown that vitamin A controls bone shape, by its control of the activity of the osteoclasts and osteoblasts. The later lectures deal with the anticalcifying and rachitogenic effect of cereals, and the action of vitamin D. This deleterious effect of cereals is shown to be due to their content of phytic acid, which can combine with calcium and prevent its absorption, thus interfering with the proper formation of bone. One of the functions of vitamin D is to facilitate hydrolysis of phytic acid to inorganic phosphate. The conclusions drawn are fully documented by experimental protocols. H. V.

RESEARCH IN MEDICAL SCIENCE. Edited by DAVID E. GREEN, Ph.D., Univ. of Wisconsin, and W. EUGENE KNOX, M.D., Molteno Inst., Cambridge. Pp. 492. New York: Macmillan, 1950. Price, $6.50.

THIS is an interesting book. The 26 articles include such subjects as research in surgery, pediatrics, psychiatry, viruses, cancer, anthropology, heart diseases, and chemotherapy. The subjects are, in general, well chosen and are presented by authorities in the respective fields. A regrettable failure to include research in modern pathology may be explainable on the basis of the biochemical and clinical disciplines of the editors.

The book is recommended for the layman as well as for the professional, but the technical terminology in many of the articles places it well beyond the scope of the nonprofessional reader. It should prove of most interest to the clinician and advanced medical student. D. C.

THE DEVELOPMENT OF GYNAECOLOGICAL SURGERY AND INSTRUMENTS. By JAMES V. RICCI, M.D., Clinical Prof. of Gynaecology and Obstetrics, New York Medical College. Pp. 594; illustrated. Phila.: Blakiston, 1949. Price, $12.00.

SUPPLEMENTARY to the Author's earlier volumes on gynecological history, *The Genealogy of Gynecology* (1944) and *100 Years of Gynecology* (1945), the Author now offers this third book on the same subject, repetition being minimized by the prominence given here to the instrument side of his subject. Illustrations of instruments abound, almost *ad fatigationem*—a guess would place the number in the hundreds and one can hardly come closer as they are neither numbered nor listed. Ten chapters follow a chronological sequence, from the Greco-Roman period through the pre-Anesthetic period. (Is there a fourth volume in prospect?) Each chapter is followed by increasingly long annotated references—in the last chapter reaching to 860. On the other hand, there is no subject index. The notes may well constitute the most durably valuable part of the work and in themselves justify the Author-practitioner's invasion of "the sacred province of the medical historian". Shades of Osler, Cushing, Sudhoff! The Author in this case has demonstrated the true devotion that he claims and doubtless his colleagues will be sufficiently edified by the results. Another practitioner who has at times invaded "the sacred province" regrets, however, that more scholarly help was not utilized in order to eliminate some of the undesirable features of this provoking volume. E. K.

PHYSIOLOGY OF HEAT REGULATION AND THE SCIENCE OF CLOTHING. Edited by L. H. NEWBURGH, M.D., Prof. of Clinical Investigation, Univ. of Michigan. Pp. 457; 78 figs. Phila.: W. B. Saunders, 1949. Price, $7.50.

A BOOK designed to help fill the long-existing gap in the literature of physiological heat and temperature studies is here presented. There are some 15 contributors, including well-known authorities.

Part I (9 chapters) is devoted to the Human Response to Climatic Environment; and Part II (3 chapters) to Clothing, a Thermal Barrier. The book attempts to describe the responses of the heat regulatory mechanism to the entire range of climatic conditions encountered on the earth's surface. While failing to complete such an ambitious project, it makes available a considerable amount of information from both military and civilian research.

This book is recommended for research workers as well as for senior students. Readers will note one difficulty, that is, a disagreement among contributors as to the units used in recording data. The book remains, however, a valuable source of information for those interested in temperature research.

I. D.

ATLAS OF ROENTGENOGRAPHIC POSITIONS. By VINITA MERRILL, while Educational Director Picker X-ray Corporation. In 2 Vols. Pp. 679; illustrated. St. Louis: C. V. Mosby, 1949. Price, $30.00.

THESE 2 volumes provide for the radiologist and his technical assistants a beautifully illustrated atlas showing all of the conventional roentgen examinations and many of the unusual. The Author has been meticulous in providing information concerning a great many of these examinations including brief descriptions of the anatomy of the parts. The various bones are illustrated in color plates as well as the individually dissected specimens. The technician, therefore, is provided with considerable information concerning the various structures in various parts of the body.

One finds very little to criticize in this book. Certain radiologists have pet positions for demonstrating disease of certain parts and some of these may not be included in this atlas; but it would be fanciful to expect to find all technical procedures employed in various special divisions of roentgenography. The important ones are in these books. The Reviewer does feel, however, that too many of the examinations have been illustrated in the horizontal posture. There are certain structures such as the paranasal sinuses which should be studied in the erect posture if at all possible, because greater information can thus be obtained. The same is true for the chest examination; in fact in the roentgen study of many parts of the body, the horizontal beam examination provides more information than the vertical beam. It is hoped that the re-publication of these volumes will include more emphasis on the value of examinations in the erect posture; however, this suggestion should in no way detract from the value of the present 2 volumes. They should be in the library of every radiologist and radiological technician.

The publisher is to be congratulated on the type of print, caliber of paper and the beautiful illustrations. It certainly sets a high standard for books of this type. E. P.

RADIOACTIVE TRACER TECHNIQUES. By GEO. K. SCHWEITZER, Ass't Prof. of Chemistry, Univ. of Tennessee, and IRA B. WHITNEY, Oak Ridge. Pp. 241. New York: D. Van Nostrand, 1949. Price, $3.25.

THIS is a guide book to laboratory work with radioactive materials. As such it is unique to date. The first 4 chapters present the practical aspects of the hazards involved and the design, construction and operation of a radiation laboratory. In Chapter V, 7 basic experiments are discussed in which the Lauritsen electroscope and the Geiger-Müller counter are described, studies are presented on beta particle characteristics, the separation of radio-elements, and the chemical behavior of micro-quantities. Chapters VI, VII and VIII deal respectively with the chemical, physical and biological applications of radioactive techniques. Appendices include a table of fundamental constants, a list of suppliers of radioactive equipment, an apparatus list and a form for writing up experiments.

The book is written in clear, readable style and covers a surprisingly large number of the problems that confront the worker who adopts radioactive methods. It will no doubt be of greatest service to those investigators who have had no previous experience in this field; but it can be read to profit by the veteran as well. A copy should be in every laboratory where work with radioactive materials is done, or contemplated. D. C.

DENTAL ANATOMY. By ROBERT C. ZEISZ, D.D.S., and JAMES NUCKOLLS, D.D.S., Univ. of California. Pp. 486; 427 ills. St. Louis: C. V. Mosby, 1949. Price, $14.00.

BASICALLY this book is a study of the morphology of individual teeth and does not include general anatomy, histology, embryology or other related subjects. However, there is some consideration of the supporting structures of the teeth as well as an experienced analysis of the correlation between tooth form, function, chewing and occlusion generally.

The aim is to give the student an idea of the structures and landmarks of the deciduous and permanent teeth, not only descriptively but also by composite illustrations both diagrammatically and by depicting teeth from 5 aspects (buccal, lingual, occlusal, mesial and distal). These composite illustrations were made from a collection of 6000 permanent teeth and an equal number of deciduous teeth, accumulated at the University of California over a period of many years. In tables on pp. 456-8 only the average diameters are given—*no range*. The Authors are competent to present the material since they both are teachers of 20 years experience in California dental schools. They show a breadth of approach which goes beyond mere tooth form when they on many occasions point out correlative factors in tooth form involved in chewing and occlusion.

Stress on the importance of the deciduous teeth as predecessors to the permanent teeth constitutes a useful functional approach. One may admire the painstaking odontologic detail of this study but wish that the Authors had concentrated less upon stereotypes and more upon the variability in size, shape and general morphology of the individual tooth. There is danger that if teeth do not conform to the average in size and shape as given in this volume, the student could easily assume that they were not normal. It would have been desirable if the Authors had hit harder the theme of "individual normal" so basic to all human morphology. C. Z.

NEW BOOKS

Ecology of Health. Edited by E. H. L. Corwin, Ph.D. Pp. 196. New York: Commonwealth Fund, 1949. Price, $2.50.

". . . the quintessence of the papers and discussions at the Institute on Public Health which was held at the New York Academy of Medicine on April 1, 2, and 3, 1947, as a part of the Centennial Celebration of the Academy."

Haemolytic Disease of the Newborn. By M. M. Pickles, D.M., Radcliffe Infirmary, Oxford. Pp. 181; 21 figs. Springfield, Ill.: Charles C Thomas, 1949. Price, $4.50.

This is an excellent monograph. Dr. Pickles is an experienced worker in this field to which she has made some fundamental contributions. The volume is authoritative, well organized, well written, well illustrated, thoroughly practical and adequately documented. The index is excellent. The Reviewer recommends this little book with genuine enthusiasm. T. F-H., Jr.

Therapeutic Exercises. By Hans Kraus, M.D., Ass't Clinical Prof. of Rehabilitation and Physical Medicine, New York Univ. College of Medicine. Pp. 336; 290 ills. Springfield, Ill.: Charles C Thomas, 1949. Price, $6.50.

This first edition presents a rational approach to the use of exercises in treatment. The illustrations are clear, accurate and useful. The first section is somewhat repetitious, but this is not without its advantages. Because of its logical basis and avoidance of long lists of complicated exercises, the book will be of value to all physicians who encounter cases of neuromuscular and joint disease and other conditions causing general debility. J. H.

Bedside Diagnosis. By Charles Mackay Seward, M.D., Royal Devon and Exeter Hospital. Pp. 372. Balt.: Williams & Wilkins, 1949. Price, $3.50.

Symptoms rather than signs or lesions form the basis for classification. Of 16 different symptoms, pain is given for 7 short chapters. Anemia and hemorrhage are allotted 70 pages, whereas Dyspnoea, Tachycardia, and Pyrexia receive shorter shrift. The volume contains much condensed information, but is inadequate for its worthy and ambitious purpose.

Congenital Anomalies of the Heart and Great Vessels. By Thomas J. Dry, Robert L. Parker, H. Milton Rogers, Jesse E. Edwards, Howard B. Burchell, and Arthur H. Bulbulian of the Mayo Clinic. Pp. 68; 80 ills. Springfield, Ill.: Charles C Thomas, 1949. Price, $4.50.

This is a brief presentation of the material assembled primarily for a convention exhibit. The monograph consists of a pictorial portrayal of nearly all of the congenital cardiac anomalies. Each anomaly is represented by a picture, roentgenogram, and a characteristic electrocardiogram. Included with the actual data are reproductions of portraits of the pioneer investigators in the field of congenital heart disease. As a visual aid this monograph will aid materially in the teaching of congenital cardiac lesions. H. V.

Partition Chromatography. Edited by R. T. Williams, Assisted by R. L. M. Synge. *Biochemical Society Symposia No. 3.* Pp. 103; 12 ills. Cambridge University Press, 1949. Price, $1.50.

Excellent in content and manner of presentation.

Angina Pectoris and Myocardial Infarction. By Heymen R. Miller, M.D., Ass't Prof. of Medicine, New York Postgraduate Medical School. Pp. 336; 63 ills. New York: Grune & Stratton, 1950. Price, $8.75.

Normal Values in Clinical Medicine. By F. William Sunderman, M.D., Ph.D., Prof. of Experimental Medicine and Clinical Pathology, Univ. of Texas, and Frederick Boerner, V.M.D., Late Assoc. Prof. of Clinical Bacteriology, Univ. of Pennsylvania. Pp. 845; 237 ills. Phila.: W. B. Saunders, 1949. Price, $14.00.

This large volume, which exemplifies how much the clinician depends today on objective information about normal values, covers in its 74 chapters far more than clinical laboratory tests. The standing of its 2 Authors and 29 collaborators is a strong guarantee of its accuracy and value. Now to keep it up to date! E. K.

Medical Clinics of North America, Symposium on Pediatrics. Pp. 1-306. Chicago Number. Phila.: W. B. Saunders, January, 1950. Price, $15.00 a year.

Brief discussions are presented on 21 different subjects, with particular reference to the Authors' personal views and experience. As in any such group of papers, there is much to approve and some opinions which are controversial. Self-demand feeding is presented in good perspective. Removal and examination of cerebrospinal and subdural fluid are well discussed, also acute and chronic nephritis, paroxysmal tachycardia, and tracheo-esophageal fistula. Useful suggestions are given for diagnosis and management of several other disorders. Many will question the use of

penicillin or other potential sensitizers in nose drops and aerosols for allergic children. Other criticizable features are the exclusive emphasis on dietary sugar in control of dental caries, omission of intrathecal streptomcin in the treatment of tuberculous meningitis, free activity in the management of Legg-Perthe's disease, late surgery for non-paralytic strabismus, and immediate transfusion of all erythroblastotic infants. On page 188, line 7, "Rh negative" is apparently meant, not "positive" as written. Errors in proofreading are common throughout. F. H.

The Chemotherapy of Tuberculosis—The Experimental Approach. By G. RAKE, *et al.* Pp. 165; illustrated. New York: Annals of the N. Y. Acad. of Sci., **52**, Art. 5, 1949. Price, $2.50.

From the Hills, An Autobiography of a Pediatrician. By JOHN ZAHORSKY, M.D. Pp. 388. St. Louis: C. V. Mosby, 1949. Price, $4.00.

DIVIDES his life into 3 parts, Development, Maturity, Decline, of 30 years each. The age of decline is not yet completed and "will probably not be fulfilled (D. V.)".

Metabolism and Function. Edited by D. NACHMANSOHN, M.D. Pp. 346; illustrated. New York: Elsevier Publishing Co., 1950. Price, $7.00.

A COLLECTION of papers dedicated to Otto Meyerhoff on the occasion of his 65th birthday.

Chemotherapy of Leukemia and Leukosarcoma. By WILLIAM DAMESHEK, M.D., MARVIN L. BLOOM, M.D., LOUIS WEISFUSE, M.D., MILTON H. FREEDMAN, M.D., and MIGUEL LAYRISSE, M.D. Pp. 54; illustrated. New York: Grune & Stratton, 1949. Price, $4.75.

THIS reprint of an exhibit presented at the American Medical Association Convention, Atlantic City, June, 1949, is an excellent picture-book story, briefly and effectively told, of the newer agencies and methods of therapy of leukemia and leukosarcoma. The Reviewer's experience is somewhat similar, but his optimism falls short of that of the Authors' in so far as the future is concerned. The achievements of modern therapy of leukemia are still tragically unsatisfactory. The use of ACTH in this field began after this volume was compiled. The book is heartily recommended to all students of leukemia. T. F-H., Jr.

Helpful Hints to the Diabetic. By WILLIAM S. COLLENS, M.D., and LOUIS C. BOAS, M.D. Pp. 135; 64 ills. Springfield, Ill.: Charles C Thomas, 1949. Price, $3.00.

L'Organisation des Os. Par P. LACROIX, Prof. à la Faculté de Medecine de Louvain. Pp. 230; 87 ills. Paris: Masson et Cie., 1949. No price given.

The Question of Lay Analysis. By SIGMUND FREUD. Translated by NANCY PROCTER-GREGG. Pp. 125. New York: W. W. Norton, 1950. Price, $2.50.

Aktive Entspannungsbehandlung. Von Dr. med. JOH. FAUST. Pp. 127; 14 ills. Stuttgart, Germany: Marquardt & Cie., 1949. Price, D. M. 6.80.

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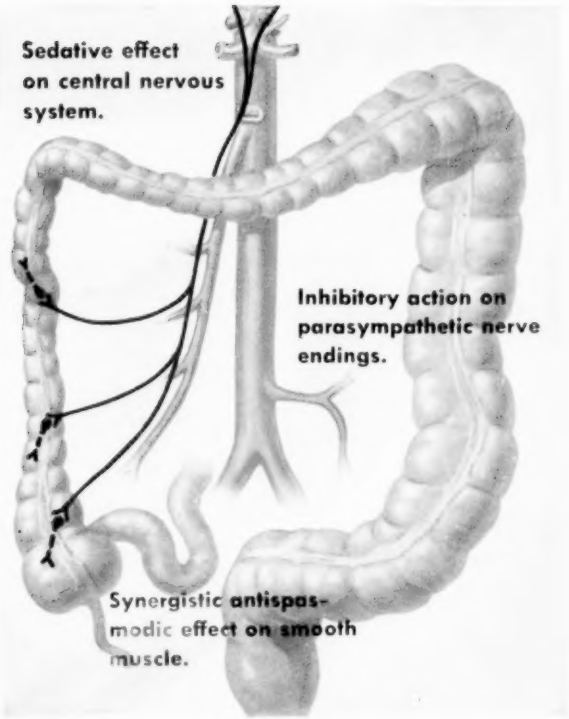
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